



NEUROENDOCRINOLOGICAL  
STUDIES IN STRESS  
EXPERIMENTAL SURGICAL  
OBSERVATIONS  
IN  
VERTEBRATES AND INVERTEBRATES

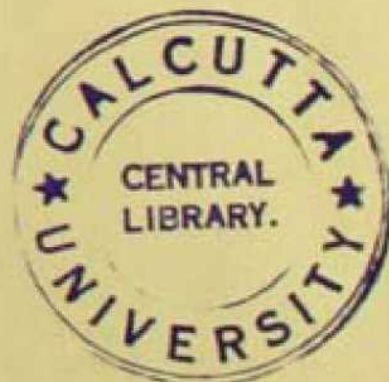
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DEPARTMENT OF EXPERIMENTAL SURGERY

R. G. KAR MEDICAL COLLEGE & HOSPITALS

AND

UNIVERSITY COLLEGE OF MEDICINE, CALCUTTA UNIVERSITY



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DEDICATED TO—

- The Sacred memory of my Father who wanted to make me a Man.
- My Mother who through hardships built up my Medical Career.
- My beloved Teachers who taught me the art and principles of Surgery and Experimental Surgery.
- My Wife who constantly inspired me throughout my Professional and Research Career.



## FOREWORD

It has been realised a long ago that the subject, Experimental Surgery, forms a vital sub-group of Surgery and must be studied separately in modern Medical Sciences. In the course of his usual work a Surgeon sometime finds an intricate problem, the answer to which can only be obtained from the pragmatic application of experimental surgical methods. So, every practitioner in Medicine is becoming increasingly conscious of the vast importance of this subject which calls for extensive research by devoted scholars. Dr. B. B. Roy has been deeply interested in the subject from a long period and has carried on research, especially on the subject of comparative study of Hypothalamo-pituitary-adrenal axis mechanism. He has published the finding of his research from time to time in different journals and now he has collected all these papers and published them in the form of a book where he has divided the subject matter of his research into three parts, *viz.* Part I deals with the adrenocortical responses in some surgical and experimental conditions; Part II deals with the brain mechanisms responsible for ACTH release in experimental burns, and Part III deals with comparative studies on stress and brain mechanism in different species.

I am sure that the fruits of his research will prove beneficial to all scholars in this field and will also be of considerable help to practising surgeons. I am really glad that more and more attention is now being paid to research on health problems in our country. That is one of the ways in which those of us who have to work in the University can repay the debt we owe to the suffering public.

SENATE HOUSE :  
24th May, 1974

DR. S. N. SEN,  
*Vice-Chancellor.*  
University of Calcutta.



## PREFACE

Following my graduation in Medicine in the year 1947 while I was working as House-surgeon in the Department of Surgery, R. G. Kar Medical College, Calcutta, under my revered teachers Dr. Subodh Dutta and Late Dr. Umapada Mukherjee, the latter one day drew my attention to the large number of cases of Peritonitis with or without acute intestinal obstruction and inspired me to undertake a systematic study of such unfortunate patients with special reference to salt balance. At his instance I earnestly took up the problem and that, in fact, marked my entry into the fascinating field of research in endocrinology with my attention specially focussed on adrenocortical function. This maiden research eventually led me to prepare a thesis, titled as "Prognostic Evaluation of Acute Intestinal Obstruction cases with special reference to Adrenocortical Changes", which was then duly commended in fulfilment of the prerequisite for M.S. Examination of the Calcutta University in the year 1949-50.

Charged with the thrill of this initial investigative work I subsequently carried on a second research project based on clinical and experimental studies, objectively designed to evaluate the role of Pituitary-adrenal-axis in burn. By and by, through extension of my work into divergent channels relevant to the fundamental topic, I became deeply interested in the subject of comparative study of Hypothalamo-pituitary-adrenal axis mechanism. Since then, I ceaselessly continued my endeavour on different parametres of the aforesaid topic and started publishing the observed results in different journals serially. The collective data of all these experimental surgical works have led me to realise that the phenomenon of adaptation to stress mechanism not only does occur in higher vertebrates like man, but significantly enough, it is evident in the lower vertebrates as well, right up to the level of fish. In the invertebrates also the neuroendocrinal mechanism does play its role by helping the animals to tide over the critical situation of life, that is to say, it regulates the mechanism of adaptation to environment and thereby upholds the theory of the survival of the fittest in universal practice.

Through these experimental works it has also been evident that the hypothalamus is not the only nodal point for controlling the pituitary but this point is obviously controlled by vast extrahypothalamic influences. It seems relevant in this connection to emphasize that for the satisfactory operation of these experimentations the help of experimental surgery had





to be immensely utilised. It is to be realised that the discipline of experimental surgery is a separate entity in the modern medical sciences and as well a vital and viable subgroup of surgery. A surgeon in course of his routine work sometimes finds an intricate problem, the answer of which can only be obtained through the pragmatic application of experimental surgical procedures. Honest research for solution of such problems can only make one's career perfect and more purposeful as a surgeon. Experimental Surgery, as such, is a vast subject and I deem it my duty to keep on record here that only certain aspects of neuroendocrinologic problems have been dealt with in this treatise.

For the sake of convenience, the treatise has been divided into three parts : Part I deals with the adrenocortical responses in some surgical and experimental conditions; Part II deals with the brain mechanisms responsible for ACTH release in experimental burns, and Part III deals with the comparative studies on stress and brain mechanisms in different species.

Before closing, I take this opportunity to express my heartfelt gratitude and deep sense of reverence to my beloved teachers, specially to Late Dr. Lalit Mohan Banerjee, Late Dr. Panchanan Chatterjee, Late Dr. Umapada Mukherjee, Late Dr. Nirode Baran Mandal and to Dr. Subodh Dutta, Dr. Amiya Kumar Sen, Dr. Amar Sen, Dr. Rudrendra Kumar Pal and Dr. Bishnu Pada Chatterjee for their scholastic guidance, sincere encouragement and critical advice at different stages which have gone a long way in building up my career not only as a surgeon but also as a humble devotee of experimental surgery. Being connected with the Department of Experimental Surgery, as it has developed to-day in my Alma Mater through my relentless efforts for more than two decades, I pay my sincerest tribute to those of my teachers whose blessings and impetus affectionately sustained me in the days of hardships and in the moments of despair. In this connection I also remember Late Dr. Hemanta Kumar Indra, Ex-Principal, R. G. Kar Medical College Hospital, and Dr. Amiya Kumar Sen for whom the department stands as it is to-day.

As stated earlier, the different works have been published from time to time in various journals and compilation of some of them to-day in a book form is but the product of indomitable desire and undaunted inspiration of Late Dr. Sailendra Nath Sen, Dean, Faculty of Medicine, Calcutta University, to whom I pay my most respectful homage. As he conceived, a book of this kind would be of profound benefit to the future students and would act as a fillip to the advanced research scholars in the field. He as





well as all other teachers of mine who are no more with us to-day, I believe, would have been much gratified to see the publication of this book. Alas! this is a misfortune that the same could not be accomplished earlier!

I express my deep sense of gratitude to our respected Vice-Chancellor, Dr. Satyendra Nath Sen, for his affectionate blessings and kind permission to get the book published by our University from the University Press. It is a proud privilege on my part to have the Foreword of the book contributed by our Vice-Chancellor, Dr. Sen.

My gratitude is extended to Dr. P. K. Bose, Pro-Vice-Chancellor (Academic), and Sri Arun Ray, Pro-Vice-Chancellor (Business Affairs and Finance), Calcutta University, regarding publication of this book.

I also extend my warm regards to Sri Prafulla Kanti Ghosh, Minister of Food and Sports, for kindly supplying me some rare books and to Sri A. K. Panja, Bar.-at-Law, Minister-in-Charge, Department of Health and Family Planning, for their constant encouragement and all possible help.

I am also indebted to Late Professors M. Gabe, B. Houssay, G. W. Harris and J. D. Green and Professors K. Lissak, Hans Selye, George Sayers, C. N. H. Long, Tadashi Miyake, I. Chester Jones, de Morsier, G. E. Pickford, M. Oliverneau, M. Weitzman, K. G. Wingstrand, J. Sterne, C. Barker Jorgensen, H. A. Bern, L. Arvy, J. W. Atz, A. Stahl, L. Martini, J. Joosse, J. J. Van Mol, and others, for their invaluable suggestions which are milestones in my research career.

I like to submit with apology that there are certain unavoidable repetitions of some references and methodology in different chapters mostly because of their overlapping nature.

Learned readers will please excuse me for not furnishing some of the figures mentioned in the text. Others may differ with me in respect of any of my views expressed in this book. I beg to be excused for that also.

Thankfully I keep on record my appreciation of the active co-operation and assistance of my student-colleagues, Dr. Dilip Kumar Misra and Dr. Ajit Kumar Dutta, in the matter of compilation of the manuscript and other relevant affairs. I am also grateful to Dr. S. Chaudhuri for his kind help in photography work.

I am deeply indebted to M/s. Masson et Cie, Prais, France, for allowing me to use some figures in this book from their publications.



(x)

I am thankful to Sri Atul Kumar Bose and Sri Gour Mohan Ghosh for their friendly help in accomplishing many of the technical works including those related to artistry, photography and typing.

Deep sense of gratitude is also extended to Sri Ranabir Das Gupta, Superintendent, Calcutta University Press and Sri Salil Banerjee of N.S.S., Calcutta University, for supervision and early publication of the book. My gratitude also extends to the Press-staff who toiled for this publication.

I am deeply indebted to the Editors of all those journals who published many of my works primarily.

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August 15, 1976.*





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# PART I

## ADRENOCORTICAL RESPONSE IN SOME SURGICAL AND EXPERIMENTAL CONDITIONS (1959)



## INTRODUCTION

Physiologists before Claude Bernard knew that a constant change in the organism leads to the stability in the organism, but Claude Bernard extended it further in his "*le milieu intérieur*." On December 17, 1857, he made the following statement (From Olmsted and Olmsted, 1952).

"The blood constitutes an actual organic environment intermediary between the external environment in which the complete individual lives and the living molecules which cannot safely be brought into direct contact with this external environment. Thus the blood contains all the elements necessary to life, elements which it obtains from outside by means of certain organic mechanisms. It then serves as a vehicle for all the influences which, coming from without, act upon the fibres of the tissues : oxygen, nutritive substances, temperature etc."

"In the blood, all the tissues are provided for the accomplishment of their functions with constant conditions of temperature, moisture, availability of oxygen, as well as nitrogenous materials, carbohydrates and salts, without which the organs cannot be nourished." The final concept of Bernard was "All the vital mechanisms, varied as they are have only one object, that of preserving constant the conditions of life in the internal environment".

These studies were further elaborated by Cannon in his work on the role of the sympathetic nervous system in maintaining a constant internal equilibrium. He used the term "homeostasis" referred to structured systems "which like the feed-back systems of the engineer, react to change and thereby result in restitution to the previous state" (Mirsky, 1953). In the first Keller Memorial Lecture (1953) Moore said about Halsted and Cannon as "Surgery had been courting science. It was Halsted who brought surgery, presentable at last, to the church for the wedding. And Cannon put science finally ready to ascend, there to accept the ring. Neither was a virgin, but the offspring has been vigorous."

After Cannon, Professor Hans Selye postulated his "General Adaptation Syndrome." His observation is that noxious agents not only produce specific damage but call forth a non-specific generalised response. He said that when organisms are exposed to noxious stimuli of sufficient intensity and duration, catabolic products are formed which lead to "Alarm Reaction." This is the first stage of General Adaptation Syndrome. The alarm reaction can be divided into two phases.

- (a) Shock phase.
- (b) Counter-shock phase.

In the shock phase there are evidences of excitation of the autonomic nervous system.

If the damage produced by the noxious agent is not very severe, then the counter-shock phase develops. In this stage there will be an enlarged and hyperactive adrenal cortex.



After the counter-shock phase there is the "Stage of Resistance" and finally the "Stage of Exhaustion."

Clinical and experimental studies with surgical interventions lead to the idea of increased adreno-cortical activity (Selye, 1938 ; Selye *et al.*, 1940 ; Reed, 1938 ; and Kinnunen, 1951).

It is my intention to study the stress responses after fractures and operations upon bones and joints, and moreover judging a patient after trauma or surgery from a surgeon's viewpoint only will not be beneficial for the patient. Endocrinological forces coming into the patient's system after these incidents must also be considered for the proper management of the patient. The pituitary, adrenals and other endocrine organs come into play, more or less, during the stress period to tide over the critical situation, for the welfare of the patient. Some of the different pathways along which stress messages travel have already been worked out. It is not my intention to deal with all the endocrinological forces that are known to work during stress. I have limited my investigations to study the adrenocortical activity and analyse some of the pathways concerned in stress.

The investigations have been carried out as follows :—

1. Adrenocortical study in different types of fractures, complications of fractures, diseases and operations upon bones and joints, and complications after operations—by eosinophil count.

2. Study of the pathways for the stress message.

3. Neurosecretion in relation to stress.

4. Study of the histamine content of the fracture haematoma and blood in stress and its relation to eosinophil cells in the blood of stressed patients.

5. The following plan has been adopted in the chapter on the hypothalamus.

- (a) Histamine concentration of the hypothalamus, anterior and posterior pituitary, median eminence and cerebral cortex in burns, intestinal obstruction and fracture has been noted.

- (b) Histamine content of the blood from the hypophysiportal vessels has been noted after stress and this has been compared to the histamine content of the blood from a peripheral vein.

- (c) Histamine concentration in the peripheral blood of hypophysectomized and adrenalectomized dogs has been studied.

- (d) Hypothalamic extracts have been prepared in different poststress days and their action on 17-OHCS secretion has been noted in the test dogs.

- (e) Experiments have been done with blood, peritoneal fluid and content of fracture haematoma from different stressed conditions to note their action on the pituitary-adrenal axis.

6. Histological study of the adrenals and the hypothalamus.



## CHAPTER I

### ADRENOCORTICAL STUDY

Shipley *et al.* (1946) found rise of urinary corticoids after surgery. Talbot *et al.* (1947) and Tompsett and Oastler (1947) also found increase in corticoid excretion after surgery. Davis and Hulit (1949) found eosinopenia in man after surgery.

Forbes *et al.* (1947) found that the rise in 17-ketosteroids after traumatic injuries in normal subjects stayed for a very short period and then there was a great depression in the excretion level. Venning and Browne (1949) could not find any parallelism between urinary biocorticoids and 17-ketosteroids in certain conditions. Forbes *et al.* (1947) and Venning and Browne (1949) explained the discrepancy in the excretion of biocorticoids and 17-ks by suggesting a twofold action of the adrenal cortex. The excretion of 17-ketosteroids reflected the androgenic function of the cortex whereas the excretion of corticoids reflected the secretion of the cortical hormone. In stress, the androgenic activity was diminished and thus 17-ks excretion was diminished; but the corticoids increased to tide over the emergency period. Venning *et al.*, found rise of urinary corticoids after trauma in well-nourished subjects. In malnourished subjects the rise was usually lesser than normal after trauma.

Postoperative eosinopenia was studied by Laragh and Almy (1948), Roche *et al.* (1950), Coppinger and Goldner (1950), Hasner and Nielsen (1951), and Rehn (1951). About 90-95 per cent of the cases showed by them had post-operative eosinopenia. The eosinopenia stayed for one or two days. Then the count rose to the pre-operative level. Laragh and Almy (1948) and Coppinger and Goldner (1950) found a correlation between the eosinopenia and the severity of the operation; but Hasner and Nielsen (1951) and Rehn (1951) did not find the correlation; but they attached prognostic significance to the eosinopenia.

Hasner *et al.* (1952) in their book on "Adrenals in Surgery", have mentioned the study of eosinophil counts in surgical patients. Pronounced eosinopenia was found in 95 per cent of the post-operative patients. The eosinopenia had relation to the severity of the operation. A secondary eosinopenia followed the primary one when the complications were severe. The post-operative eosinopenia was an expression of increased adrenocortical function.

They studied the 17-ks and corticoid excretion after surgical intervention. The levels were high after operation. Hasner *et al.* (1952) said "In 100 out of 150 cases we found a significant increase of the 17-ks excretion. In a few of the remaining 50 cases the failing rise may possibly have been only apparent. The corticoid excretion and the BRS (DHI)



excretion showed pronounced increases in practically all cases. The increased post-operative excretion of steroids is presumably independent of age, sex and nature of the disease, qualitatively as well as quantitatively, and with regard to the time of occurrence, but to a certain degree dependent on the extent of the operation."

Wound infection, phlebitis, infarction, ileus, severe shock, and peritonitis lead to a secondary rise after the primary one.

Browne *et al.* (1950) observed the following in fracture :—

1. In a case of fracture (femur and tibia) the urinary glucocorticoids rose to 200 glycogenic units per day. This excretion level was found after injection of about 120 mg. ACTH in normal human beings. This high value with some fluctuations persisted for about 25 days.

2. In another case of fracture femur and tibia, the urinary glucocorticoid value ranged from 200 to 300 glycogenic units per day and this continued for 20 days.

3. In the same paper another case has been mentioned, 3 months after fractured jaw and fractured femur with wound infection and temperature of 101 F. The urinary corticoids were within normal range. We cannot call this normal, because in such condition of the patient this value is inadequate for the person.

Roche, Hills and Thorn (1950) found changes in the blood eosinophil level after major surgical operations. Within 24 to 48 hours after a major operation, the eosinophils almost completely disappeared from blood provided the adrenal cortical activity was normal. The levels of the eosinophils rose on the second and fourth post-operative day, and the patient's clinical condition improved. The eosinopenia in the first 24 to 48 hours after operation was an evidence of increased adrenal cortical activity. If in the first 24 to 48 hours after operation the eosinophil count was normal or high it suggested adrenal cortical insufficiency provided there was no allergy. The pre and post-operative ACTH tests gave a good idea about the functioning capacity of the adrenal cortex.

In 1953, Franksson and Gemzell investigated 4 cases of lacerations or fractures. These patients had no other endocrinological disorder. They studied blood levels of 17-hydroxycorticosteroids, eosinophils, urinary 17-ketosteroids, haemoglobin, and erythrocyte sedimentation rate. The blood levels of 17-hydroxycorticosteroids in young normal individuals vary from 4—10 microgram per 100 c.c. of whole blood (Nelson and Samuels). In two injured cases the values were 3.0 and 5.0 microgram which are low figures. These were high when the shock phase of the patients was over. The reason of low value of 17-hydroxycorticosteroids during the shock phase may be that shock produces anaemia or anoxaemia in the pituitary gland and it stops the production or secretion of ACTH. They also found rise of 17-hydroxycorticosteroids in the peripheral blood after surgery.

Franksson *et al.* (1954) studied 7 cases of traumatic injuries. Two of these patients with shock had a low blood level of 17-hydroxycortico-



steroids immediately after trauma. This value was high on the next day when shock diminished. In the remaining 5 cases, the value increased immediately and remained high for about 38 hours. The values then dropped to subnormal level and remained so for twentyfour hours. After this there was a second elevation, of course much less than previous one and this stayed for 48 hours. After this the values became normal. They found the rise of blood 17-hydroxycorticosteroids in other surgical cases.

Franksson and Gemzell (1955) found pre-operative rise in the level of plasma steroids and this they ascribed to psychic tension before operation.

Nicholas *et al.* (1954) studied 8 patients undergoing operations upon bones and joints. In less severe operations, the urinary neutral 17-ketosteroid levels did not rise except in case 4. In this case there was a small rise 48 to 72 hours post-operatively and it persisted only for one day. In more severe operations, the urinary 17-ketosteroid level rose in 2 cases within 24 hours and in one case within 48 hours. In the 4th case getting cortisone from 24 hours post-operatively the 17-ketosteroid level dropped sharply. A sustained post-operative rise in urinary 17-ketosteroid level was seen in a patient who had wound complication in the post-operative period. It is seen that eosinophil changes and urinary electrolyte changes are characteristic following surgery but the level of urinary 17-ketosteroid level varied. In 3 of the eight patients studied by these authors there was no elevation of urinary 17-ketosteroids during the course of the illness. Nicholas *et al.*, sum up by saying that "The 17-ketosteroid level, however, does not constantly mirror this evidence of increased adrenocortical function post-operatively".

Hardy and Ravdin (1952) studied some physiologic aspects of surgical trauma. They studied daily urinary excretion of corticoids and 17-ketosteroids in six patients. There was increase in corticoid excretion within 24 hours. This increase stayed for one to six days and the average was about 4 days. After this period of rise, there was a temporary fall below the level found pre-operatively. The urinary excretion of corticoids reflected post-operative adrenocortical activity more correctly than the 17-ketosteroid excretion. This was in full agreement with Cope *et al.* (1943), Forbes *et al.* (1947), Perry *et al.* (1949), Venning and Browne (1947), and Stevenson *et al.* (1944). Anaesthesia, operation and fever lead to a fall in the eosinophil count.

Hardy, Richardson and Dohan (1953) studied urinary corticoid excretion in 4 patients and 17-ketosteroid excretion in 5 patients after major operations. They correlated these with nitrogen, creatinine and electrolyte metabolism. Total eosinophil counts were also included in the investigation. Corticoid excretion increased in all 4 patients studied. 17-ketosteroid excretion increased in 3 of 5 patients.

There was inverse relationship between corticoids and eosinophils. As the eosinophil count was low, the corticoids were high. This inverse relationship was however not maintained between eosinophil counts and 17-ketosteroids. Therefore the observations of Hardy *et al.* (1953) and Nicholas *et al.* (1954) fully agree regarding the 17-ketosteroid levels in the post-operative period.



Moore in 1953 and 1954 divided the surgical convalescence into the following phases :—

### Phase I—Adrenergic-Corticoid Phase :—

Clinically the patient shows increased adrenomedullary activity in this phase—there is increased pulse rate, diminished pulse pressure, peripheral vasoconstriction, sweating and increased blood sugar level. Spinal anaesthesia, intravenous barbiturates, and drugs which interfere with normal autonomic transmission modify this phase, either decreasing its intensity or delaying the excitement phase.

There is a fall in eosinophil count which may start pre-operatively, the fall continues during anaesthesia, and it is zero or near zero at the conclusion of the operation. The eosinophil count may remain low for two to five days depending upon the severity of the operation, or if there is post-operative infection or pain. If there is no complication, the eosinophil count is high by the 3rd or 4th day and the count is higher than the pre-operative level. This is known as backswing overshoot. This is a characteristic phenomenon of the second phase of convalescence. Backswing overshoot may not be found in many patients.

The urinary excretion of steroids is increased on the day of the operation. After that, the urinary 17-ketosteroids come to a below-normal level. The 17-ketosteroids may not increase always after trauma but 17-hydroxycorticoids show changes always after it. The urinary excretion of 17-OHCS is normally from 5 to 10 mg. per 24 hours. This may go up from 15 to 25 on the first day after a hernia operation under spinal anaesthesia. With severity of the trauma there is rise of 17-hydroxycorticoids. After severe intraperitoneal trauma the level varies from 35 to 40 mg. per day.

In fracture femur, the highest value was 50 mg. per day.

Hardy (1958) states that the hydrocortisone secretion per minute has been measured in man during operation by collecting left adrenal vein blood at laparotomy. In the adrenal vein blood, the free hydrocortisone level was 224 gamma per cent and in the peripheral blood it was 24 gamma per cent. During operative stress the hydrocortisone output was 77 gamma/min. and it was 24 gamma/min. in the resting stage.

Moore says that the wound is an "endocrine organ" and circulating substances from it maintain or intensify the response. He further states "clinically minor response elicited by a wound in the anaesthetised extremity of a paraplegic patient suggests that the neural pathway centrally is more important than the blood stream in relating the wound to the response".

He has mentioned "It appears to us that the weight of evidence, based on human studies, favours the concept that there is an actual increase in the secretory activity of the pituitary and the adrenal cortex after injury and that subsequently these factors are subject to diminution or withdrawal".



## Phase II—Corticoid withdrawal Phase :—

The eosinophil count backswings to a level which is higher than its resting value. This backswing overshoot stays only for a day, or a fraction of a day. In extensive trauma, as for example burns, where the eosinophil count is low for a long time, the backswing overshoot stays for a longer period at a higher level. Prognostically this is good.

Rise of eosinophil count 3 or 4 days after severe trauma is good. A continuous low value signifies a continuous tissue pathology. The urinary excretion of 17-OHCS comes down to a decreasing value. This lowered 17-hydroxycorticoid value parallels the decrease in nitrogen excretion rate; in fracture cases 17-hydroxycorticoid value comes down to normal but the nitrogen excretion rate is high and this persists for a long time.

Moore finds a good correlation between the findings in this phase and the findings when exogenous ACTH is suddenly withdrawn from a normal person.

Steenburg (after Moore, 1954) has shown that the normal free blood steroid is from 5 to 12 gamma per cent. This value reaches to 30-60 gamma per cent within two to six hours depending upon the type of trauma. The value then falls down to normal or is above normal within 24 hours after surgery.

He has further shown that trauma gives rise to a higher blood level of steroid than is found after an injection of maximum dose of ACTH before trauma; and moreover a dose of ACTH before trauma will lead to a blood steroidal level which is lower than that achieved by a dose of ACTH after trauma. Thus it is evident that trauma alters the metabolism of 17-hydroxycorticoids.

Steenburg and Ganong (from Moore, 1954) "have shown that adrenalectomized dogs given a constant dose of cortisone will exhibit a higher blood level of free 17-OHCS after an anaesthesia or an operation than they will under precisely the same circumstances without the trauma or anaesthesia. This would suggest that constant dose does not mean constant blood level when trauma is superimposed on the adrenalectomized hormone treated animal."

## Phase III—Spontaneous anabolic phase :—

The eosinophil count is normal in this phase. The urinary 17-ketosteroid excretion is low. Moore suggests that growth hormone is an important endocrine force in this phase.

## Phase IV—Fat gain phase :—

During this phase, the eosinophil count becomes normal. The steroid excretion in the urine becomes normal. In the previous stage *i.e.*, the spontaneous anabolic phase, the 17-ketosteroid is low in the urine, but in this phase, the value comes to normal.

In the third and fourth phases dietary calories, nitrogen, essential amino acids, and vitamins are required for spontaneous anabolism and for proper deposition of fat.



Nicholas and Wilson (1953) studied adrenocortical response in operative procedures upon the bones and joints. Their study was based on eosinophil counts. They confirmed previous observations that after operation there was a drop in eosinophil count. The drop persisted for 3 to 4 days after which the count rose to normal or above normal levels. If there were complications regarding the wound or the patient, the eosinophilia persisted. In patients with recent fractures there was eosinopenia. When the fractures were reduced and fixed, there was eosinophilia. They studied six cases with acrylic hip prostheses. The eosinophil count, after the initial eosinopenia and the rebound in the post-operative period, quickly came down below the admission level and was low for 35 days. Five patients in whom large metallic appliances *e.g.*, Kuntscher nails or large trochanteric plates were used, the eosinophil counts were low for as long as 5 weeks. But in four patients with fresh fractures of the neck of the femur treated by reduction and internal fixation, there was a rapid rebound to persistent eosinophilia.

Moore *et al.* (1955) studied urinary excretion of 17-hydroxycorticoid, and associated metabolic changes, in cases of soft tissue trauma of varying severity and in bone trauma.

Case 9—got multiple fractures (right tibia and fibula, left radius and ulna). The fracture of tibia and fibula was compound in nature. Eosinophil count was low for 3 days. After that it gradually rose to normal level. There was no overswing detected in the count. There was no abnormality in 17-ketosteroid excretion. The 17-hydroxycorticoid excretion was elevated for 2 days. After that it returned to normal. Closed reductions were done under local anaesthesia.

Case 10—had a fracture of right femur for which intramedullary rod was introduced under pentothal and ether anaesthesia. The age of the patient was 17 years. The eosinophil count stayed low for only 3 days. After that it returned to slightly higher level than that observed subsequently. The urinary excretion of 17-ketosteroid was within normal range. The 17-hydroxycorticoids in the urine was 25 mg. per 24 hours on the day of operation. The next day it was over 50 mg. per 24 hours. After that the excretion was normal except on the 14th post-operative day when there was an elevation to 14 mg.

The 3rd case is case 11—The patient had an intracapsular fracture of the right femoral neck. Smith-Petersen nail was inserted under pentothal ether anaesthesia. On the day of trauma and the day of operation the eosinophil count was low. It then gradually rose to about 120 per c.mm. There was no alteration in urinary 17-ketosteroid excretion. The 17-hydroxycorticoid rose to 11 mg. per day on the next day after operation. Afterwards it was within the range of 5 mg. per day.

Moore (1955) said that within one minute after trauma the corticoid level in the adrenal venous blood rose to a hundred times the normal. Adrenalectomized dogs injected with cortisone had a high blood corticoid level. After ether anaesthesia the level rose further and ether anaesthesia with trauma gave rise to an increase in the blood corticoid level. His idea about the rise was whether this was due to the failure by the liver to



inactivate corticoids. He further stated that the blood corticoid response was abolished by hypothermia in dogs or man. Warming after hypothermia was followed by a late response.

Thorn, Jenkins and Laidlaw (1953) did not find any increase in urinary 17-hydroxycorticosteroid when normal men were exposed to 4°C for four hours.

Eik-Nes (1955) investigated the response of the adrenals and plasma 17-hydroxycorticosteroids in dogs exposed to cold. He used mongrel dogs of 12-24 kg. and anaesthetised them with intravenous sodium pentobarbital 35 mg/kg. The animals after femoral artery canulation were kept in the cold room at 5°C. There was no elevation in the plasma 17-hydroxycorticosteroids for over 6 hours though the rectal temperature had a good fall. It was suggested that both production and metabolism of 17-hydroxycorticosteroids were diminished when the body temperature fell. Cortisol and ACTH had no preventive action on the fall of the rectal temperature at 5°C.

Nelson, Egdahl and Hume (1956) have studied the 17-hydroxycorticosteroids in the adrenal venous blood of dogs. The dogs were exposed to 10°C for 1 to 33 hours from the 3rd to the 9th postoperative day. There was no significant increase above the basal level. ACTH administration, warming following cold exposure and surgery gave rise to increased value. Selye and Schenker (1938) demonstrated that exposure to cold environment was a stress to adrenalectomized rats; but the dogs in this study failed to show increased corticoid output, meaning thereby that the dog is less sensitive than the rat at 10°C and the basal secretion of 6 mg. per 24 hours in the dog in this circumstance was quite sufficient to prevent pituitary stimulation.

Gray (1955) mentioned that the anabolic and catabolic changes after trauma were due to the secretion by the adrenal of different proportions of the hormones.

Sydnor and Sayers (1954) studied blood and pituitary ACTH in intact and adrenalectomized rats after stress. The stresses were :—

- (a) Exposure to ether.
- (b) Exposure to ether followed by a standardised scald.
- (c) Continuous ether anaesthesia and rapid ex-sanguination from the abdominal aorta.
- (d) Continuous ether anaesthesia and repeated bleeding at 4 minute intervals.

	Concentration of ACTH in the blood—milliunit per 100 ml.
1. Nonstressed intact rat. ..	less than 0.5
2. One week after adrenalectomy ..	3.2
3. Two minutes after a stressful stimulus in the intact rat ..	1
4. Same stress as in (3) but in adrenalectomized rats. ..	2 to 3 times increase
5. Six minutes after ether anaesthesia and a standardized scald in the intact rat ..	Not detectable.
6. Six minutes after ether anaesthesia and a standardized scald in adrenalectomized rats ..	High concentration.
7. Ether & ex-sanguination ..	2.6



Concentration of ACTH in the  
blood—milliunit per 100 ml.

8. "The blood ACTH titer of the adrenalectomized rat similarly stressed is not significantly different from that of the adrenalectomized rat exposed to ether alone."

Sayers and Burks (1955) studied blood ACTH during ether anaesthesia in adrenalectomized rats. There was a fall in the level of circulating ACTH gradually. This was not due to exhaustion of the adeno-hypophysis of ACTH because the ACTH concentration in the adeno-hypophysis of adrenalectomized rats subjected to ether anaesthesia for 30 minutes was the same as that found in the hypophysis of adrenalectomized animals anaesthetised for 2 mins. According to them there are two possibilities :—

"(a) CNS excitation during initiation of ether anaesthesia accelerates release of ACTH. As anaesthesia progresses the CNS excitability is reduced to a normal or less than normal level and paripassu ACTH discharge is reduced to a pre-anaesthetic rate.

(b) The postulated neurohumoral secretory mechanism in the hypothalamus is exhausted after prolonged excitation."

Sandberg *et al.* (1954) said that induction of anaesthesia and handling of tissues modify blood steroidal level in surgical procedures. Before operation, spinal anaesthesia had little effect on the plasma 17-hydroxycorticosteroid level. With general anaesthesia, there was an increase of it in most cases. After operation the rises were similar with any type of anaesthesia. The increase in 17-hydroxycorticosteroids was proportional to the duration and severity of the operation. They said, "The postoperative rise probably is the result of the continued presence of damaged tissues." The adrenals of the operated patients were submaximally stimulated because after ACTH there was further rise. They further stated "Factors other than adrenal cortical stimulation play a part in raising the steroid concentrations during and subsequent to surgery"—Moreover "There is a combined effect of increased production and decreased removal."

Tyler *et al.* (1954) proposed that the high level of 17-hydroxycorticosteroids after surgery was due to :—

- (a) Increased adrenal secretion of these steroids and
- (b) Diminished hepatic removal.

Sayers (1950) said, "It is to be hoped that observations designed to evaluate the effect of nutrition and liver disease, variables which are known to influence steroid hormone metabolism, will be included in these studies."

Brown *et al.* (1954) studied 12 patients with liver disease and 11 normal subjects. The persons were infused with I.V. hydrocortisone and the plasma levels and urinary 17-hydroxycorticosteroids were measured. A similar type of study was done with tetrahydrocortisone. They found, "The rate of disappearance of hydrocortisone was inversely proportional to the degree of liver damage as measured by BSP retention while tetrahydrocortisone disappeared at a much more rapid rate which was independent of liver function." According to them the high level of 17-hydro-



hydrocorticosteroids during stress was due to increased adrenocortical action and diminished removal by the liver.

Bonomo *et al.* (1954) incubated cortisol in vitro with the liver of normal and stressed rats. Stress leads to considerable diminution in the degradation of this corticoid by liver tissue. When the hormone was added to the liver tissue in vitro, the destruction of cortisol in the liver paralleled its various metabolic actions.

Bonomo *et al.* (1954) suggested that there was a true utilization of hormone for metabolic work. Selye (1956) warns, "It must be kept in mind, however, that under such in vitro conditions it is technically very difficult to appraise hormone utilization caused by the superimposition of stress since the tissue is placed under virtually maximal stress, any way by being taken out of the body and prepared for metabolic study."

Selye (1950) in "Stress" discussed the morphological evidence of liver damage during alarm reaction.

Mann and Lemonde (1951) found marked reduction of hepatic function as detected by bromsulfaphthalein test in rats subjected to various stressors.

Thorn *et al.* (1953) mentioned the change in the level of circulating eosinophils and the urinary excretion of 17-hydroxycorticosteroids after abdominal hysterectomy in a patient with multiple uterine fibroid. There was total eosinopenia, and a high level of urinary 17-hydroxycorticoids was found during and following surgery. They further said, "That a change in the total 17-hydroxycorticoid excretion is a more sensitive indicator of adrenal activation following ACTH administration than is the alteration in urinary 17-ketosteroid excretion."

Birke, Franksson and Plantin (1955) studied 18 patients—12 males and 6 females. The ages of the males ranged from 32 to 61 years, those of females from 24 to 72 years.

Partial gastrectomy for duodenal or gastric ulcers	..	10 patients
Cholecystectomy	.. ..	4 patients
Segmental intestinal resection for cancer	.. ..	3 patients
Nephrolithotomy	.. ..	1 patient

In all the above patients the post-operative period was uneventful. Shock, excessive haemorrhage and embolism were not found. The various 17-KS were studied both in pre and post-operative period. Corticoid excretion was also studied in 10 patients.

	Mean Post-operative rise
Dehydroepiandrosterone	.. .. 210 percent
Androsterone and etiocholanolone	.. .. 20 percent
Total 17-KS	.. .. 45 percent
17-keto-11-oxymetabolites	.. .. did not increase immediately after surgery
Reducing corticoids	.. .. 130 percent

5 patients who had low 17-KS excretion before operation did not show any marked rise after operation.



	Days after operation at which the normalcy in the levels was reached
Corticoids	3rd to 5th day
Androsterone and Etiocholanolone	Within 3 days
Dehydroepiandrosterone	7-9 days
17-Keto-11-Oxy-Steroid metabolites	9-14 days

They said, "The tendency of the 11-oxy-17-ketosteroids to increase about 4 to 11 days after the operation may be interpreted as implying that the adrenal secretion of biologically active steroids was still raised but that the body's requirements of these steroids were less than before and thus Franksson *et al.* (1954) were able to report an increase in 17-hydroxycorticosteroids in the blood at this time, but they did not discuss this finding."

Dehydroepiandrosterone showed to be a good index of increased adrenocortical activity in acute stress.

Paaby (1956) studied the chemistry and metabolic fate of 17-ketosteroids and more specially of dehydroisoandrosterone. The investigations confirmed that the excretion of dehydroisoandrosterone was a more valuable guide for the evaluation of the adrenocortical activity after surgery than the excretion of total 17-ketosteroids.

Sayers (1950) cautioned the use of urinary 17-ketosteroids as an index of increased adrenocortical activity because he could not find any correlation between the 17-ketosteroid excretion and the adrenocortical activity as judged by other measures.

The effect of trauma on corticoids was studied in dogs by Hume and Nelson (1954), Sydnor (1954) and in man by Barnes (1953), Bland (1953), Cope and Hurlock (1953), Feldthusen *et al.* (1953), Schreier and Weiser (1953), Thorn *et al.* (1953), Tompsett and Smith (1954). The effect of trauma and haemorrhage on corticoids was studied by Hume and Nelson (1954) in dogs.

The effect of trauma on 17-KS excretion in urine was studied by Feldthusen *et al.* (1953), Fellingner *et al.* (1953), Gray *et al.* (1953), Quijano *et al.* (1953), and Tompsett and Smith (1954).

Eosinophil count was studied in trauma by Bland (1953), Feldthusen *et al.* (1953), Gallico and Pizzetti (1953), Grassi *et al.* (1953), Gray *et al.* (1953), Hayes (1954), Minet *et al.* (1953), Miraglia (1951), Quijano *et al.* (1953) and Thorn *et al.* (1953a, 1953b). Eosinophil count after trauma was studied by Alvarez (1953) in rats.

Trauma leads to increase in corticoids in man and this was found by Bongiovanni *et al.* (1954), Cope and Hurlock (1954), Howard (1954), Llaurodo (1954, 1955), Testini and Buonsanto (1953), Tramontano (1953).

Trauma leads to fall in the circulating eosinophils as found by Feldthusen and Lassen (1954), Goldman *et al.* (1953), Howard (1954), Palomba and Fresu (1954), Palomba *et al.* (1952), Sas and Boros (1954).

Howard *et al.* (1955) studied whether adrenal insufficiency was found secondary to combat stress. Insufficiency could not be found. The adrenal responded well to the stress of the combat. Howard *et al.* (1954) studied corticosteroid excretion in 20 severely injured casualties. "In



no casualty did the corticosteroid excretion reach the maximal value of 18.7 mg. per 24 hours as demonstrated by one of the front-line soldiers. In general, the response to acute danger appeared to be comparable to the response to major physical trauma." The 17-KS excretion was normal in most of the soldiers.

Elmadjian (1954) found marked increase in 17-KS excretion on many occasions in soldiers under heavy fire. In seriously injured soldiers the blood eosinophils dropped markedly, but 17 soldiers in the front line did not show fall of eosinophils below 71.

Elmadjian (1955) studied adrenocortical function of combat infantrymen in Korea. He found that in the acute state, there was increased steroid excretion and increased protein catabolism, whereas in the chronic state the adrenal cortical function was dulled as judged by the steroid measurement and there was no protein catabolism. In the chronic state though the adrenal cortex was nonresponsive regarding the 17-KS and PS, it was responsive as evidenced by electrolyte data, after ACTH stimulation. He has further said, "The adrenal cortex in the first stages of stress, produces  $C_{21}$  steroids in large amounts with some  $C_{19}$  components. As the stress condition continues, either the adrenal ceases to produce increased amounts of  $C_{21}$ 's with an increment in favour of  $C_{19}$ 's or the  $C_{21}$ 's are rapidly converted to  $C_{19}$ 's in their intermediary metabolism. If we tie in the fact that there was in the acute stress increased nitrogen excretion, while the nitrogen excretion in the chronic stress situation was low to normal, it is not difficult to observe the similarity of the above suggestions to the previous hypothesis of "S" and "N" hormones of Albright (1942-1943) and Browne (1945)."

From the above it is seen that much work has been done about adrenocortical responses after operations ; but very few work is there about the adrenocortical responses after different types of fractures and their complications and operations upon bones and joints. Nicholas *et al.* (1953) studied the adrenocortical responses after fractures and operations upon bones and joints ; but they did not deal with the multiple fractures and also some of the other complications arising from fractures. Moreover they did not find eosinopenia during anaesthesia. In the present study eosinopenia has been observed during anaesthesia. In some cases the eosinopenia started before anaesthesia. I consider this to be due to psychological tension on the part of the patient. The period up to which the hyperfunctioning condition of the adrenal cortex stays in a particular orthopaedic operation, *e.g.*, intramedullary pinning is varied and not in consonance with the work of Nicholas *et al.* (1953) and Moore *et al.* (1955). Nicholas *et al.* (1953) found prolonged hyperfunctioning state of the adrenal cortex, whereas Moore *et al.* (1955) found short lasting hyperfunctioning condition as judged by the eosinophil count and urinary excretion of 17-hydroxycorticoids. Finding therefore that a co-ordinated study has not been done in different phases of orthopaedic operations, I started my investigations to enquire into the adrenocortical response in these conditions. The materials for study also include different types of fractures and their complications.



### Criterion of Adrenocortical Secretion :—

Adrenocortical steroids of the 11-oxysteroids type give rise to eosinopenia (Long, 1947 ; Hills *et al.*, 1948 ; Recant *et al.*, 1950). Eosinopenia is found after irritation of the sympathetic nervous system and also by adrenaline, sympatol, veritol, suprarenin, and ACTH (Hills *et al.*, 1948 ; Recant *et al.*, 1950 ; Rehn, 1951). Luft *et al.* (1950) found that noradrenaline had not the eosinopenic effect as adrenaline had got. Different types of stresses are accompanied by eosinopenia (Dalton and Selye, 1939 ; Selye, 1946, 1947 ; Hills *et al.*, 1948, and others as reviewed before).

The cause of eosinopenia under these circumstances can be discussed under the following heads.

1. **Inhibition of the production or release of the eosinophils from the bone marrow.** Durgin and Meyer (1951) thought that it was due to inhibited manufacture from the bone marrow. It had been found that prolonged treatment with adrenal hormones in adrenalectomized rats leads to marrow eosinopenia, but in acute experiments with adrenal cortical steroids or ACTH, the fact was not as stated above. By the time at which peripheral eosinopenia was marked, there was no change in the eosinophils in the marrow (Aschkenasy, 1952 ; Best and Samter, 1951 ; Finch *et al.*, 1951 ; Godlowski, 1948 ; Rosenthal *et al.*, 1950).

2. **Redistribution of eosinophils from blood to certain organs.** Spain and Thalheimer (1951) found after counting eosinophils in the peripheral blood and spleen of mice that the eosinophils migrated to the spleen after a single large dose of cortisone. Fruhman and Gordon (1952) found after cortisone administration to rats that splenic eosinopenia occurred similar to blood eosinopenia. Splenectomized animals as well as normal animals showed peripheral eosinopenia after cortisone, epinephrine or stress (Dury, 1950 ; McDermott *et al.*, 1950). Solomon and Humphreys (1951) studied arteriovenous differences in the eosinophil count in dogs after ACTH or epinephrine. They could not find any difference in the arteriovenous eosinophil count. This proved that redistribution of eosinophils from blood to spleen did not occur.

3. **Eosinophil cell destruction :—**

Josey and Lawrence (1932), and Godlowski (1951) suggested previously that this could be a factor for eosinopenia but no direct evidence was put forth.

As Gordon (1954) said "The predominant action of chronic treatment with 11-oxycorticosteroids on the marrow structures is to increase the percentages of nucleated erythrocytes and to lower the concentration of eosinophilic elements. Evidence is presented supporting the concept that eosinophilic leucocytic destruction is a generalised phenomenon, enhanced by adrenocortical factors and by stress."

Simms *et al.* (1951) agreed with Dougherty and White that excess adrenal corticosteroids acted by destroying the circulating blood eosinophils and



also they were inhibiting the production for release into the circulation.

Esselier *et al.* (1954) said that the eosinopenic effect of the glucocorticoids was actually not due to the direct eosinolytic mechanism.

### Adrenaline and ACTH tests :—

The adrenocortical function and the adrenocortical reserve have been studied by the eosinopenia induced by ACTH and adrenaline (Thorn and Forsham, 1950 ; De Fossay and Deltour, 1950 ; Coste *et al.*, 1950 ; Roche *et al.*, 1950 ; Rehn, 1951 ; Fisher and Fisher, 1951). If the fall in the eosinophil count exceeds 50 percent, it indicates a normal cortical function and reserve ; if the fall is less than 50 percent, it means reduced cortical function and reserve. Roche *et al.* (1950), De Fossay and Deltour (1950), Hasner and Nielsen (1951), Rehn (1951) and Hasner *et al.* (1952) studied the ACTH and adrenaline tests on surgical patients. Eosinopenia can be produced in patients with Addison's disease after injection of large doses of adrenaline. Therefore Coste *et al.* (1950), De Fossay and Deltour (1950), Roche *et al.* (1950) could not lay any importance to adrenaline eosinopenia as a definite measure to detect adrenocortical function. ACTH eosinopenia exceeding 50 percent showed a normal adrenocortical function but no importance could be ascribed to falls less than 50 per cent, or a rise (Laroche and Tremoliers, 1950 ; Videbaek, 1951).

Hasner *et al.* (1952) said, "During the post-operative period—after return of eosinophil count to the pre-operative level—both ACTH and adrenaline tests more often gave a fall less than 50 per cent than did the pre-operative tests. The cause of this is not evident either. Roche *et al.* (1950) adhere to the view of an "adrenal pituitary inertia" during this period. However the pronounced eosinopenia occurring in association with post-operative complications to a certain degree though not definitely, denies this hypothesis. It is more reasonable to presume that after the preceding stress a larger test dose is required to obtain the same reaction (in 3 instances the test was, however, repeated with 50 mg. ACTH with the same result)."

Laidlaw (1954) said that they studied two bilaterally adrenalectomized patients. The patients did not get cortisone. These patients did not respond to ACTH post-operatively ; but these patients had fall of eosinophil cell count greater than 50 percent after epinephrine.

Baker (1954) studied one such patient. The patient was having ACTH for 5 weeks. During this time there was no change in the urinary steroid excretion (oestrogens, formaldehydogenic steroids and 17-ketosteroids) and in the circulating eosinophils. This proved that there was no adrenal cortical rest tissue. In this patient epinephrine eosinopenia occurred before and after ACTH.

Rawson (1954) studied an adrenalectomized patient with ACTH. There was no post-ACTH change. However the patient was pulling through without cortisone or any other replacement therapy. He further



said "I should like to comment on Dr. Baker's comments concerning the adrenalectomized humans treated with ACTH. In the Department of Pathology at Memorial Hospital it has been observed that the incidence of aberrant adrenal tissue is in the neighbourhood of 25 per cent. Such a situation may account for some of Dr. Baker's observations."

Gordon (1954) commented on the epinephrine eosinopenia. In the mouse and in the rat, epinephrine gave rise to eosinophilia rather than eosinopenia, if the adrenals were completely removed. If a portion of the adrenal tissue was left behind or aberrant adrenal cortical tissue was present, epinephrine gave rise to eosinopenia. Studies made by Thevathasan and Gordon showed that epinephrine in adrenalectomized rat gave rise to eosinophilia but if the animal was given 2 ml. adrenal cortical extract before, then epinephrine 4 hours after did not give rise to any eosinophilia, rather in some cases actual eosinopenia occurred. This indicated that, for epinephrine eosinopenia to occur in the adrenalectomized rat, at least some cortical hormone must be present.

Further, regarding the epinephrine eosinopenia, Speirs (1954) added to the discussion by saying.

1. Local destruction of eosinophils by epinephrine did not occur either in vitro or in vivo experiments. This invited the possibility that either some factor or organ was necessary for epinephrine to act on eosinophils.

2. Epinephrine was injected subcutaneously into adrenalectomized mice and eosinophil changes were noted. Extra salt from the drinking water was then removed. The animals which showed epinephrine eosinopenia survived well without the extra salt; but the animals showing eosinophilia died. Speir's conclusion was that surviving animals had adrenal rests or remnants; but the second group did not have any such and so these animals died. This was finally proved by demonstration of adrenal cortical rests or remnants in these mice which survived and showed epinephrine eosinopenia. Thus clinically in human beings epinephrine eosinopenia in some of the adrenalectomized patients could be explained on the basis of adrenal cortical rests.

3. In adrenalectomized mice ACTH eosinopenia was not found always, nor the fall was so great as found after epinephrine. Epinephrine was followed by a refractory period, but ACTH was not followed by such a refractory period. Therefore epinephrine action could not be explained solely by the ACTH release. "Our data has led us to believe that ACTH is involved in the synthesis of oxycorticosteroid hormone, but epinephrine is necessary for its sudden release."

Assessment of the adrenocortical activity in the present study has been done with the help of eosinophil cell counts. This method is not expensive and can be repeated many times and if it is done carefully, it gives good and consistent results. Different types of orthopaedic cases have been studied. Cases treated with blood stimulants e.g., liver extract, folic acid, iron etc., have been excluded. Similarly patients suffering from parasitic infections, allergy, blood diseases, asthma etc., have been excluded from this investigation.



**Method of examination :—**

Randolph's method for eosinophil count has been adopted. As there is diurnal variation of the eosinophil count in the same man, the venous blood has been collected at the fixed morning hour of the day in all cases.

Total number of cases studied—344 including the controls.

**The types of cases including the controls are as follows :—**

Types	Number
Control cases ..	46
	healthy persons
Anaesthesia ..	87 Cases
	These patients have been taken from the group with operations
Cases without post-operative complications ..	65
Cases with post-operative complications ..	22
Simple fracture ..	34
Multiple fracture ..	20
Compound fracture ..	28
Compound fracture and gas gangrene ..	6
Compound fracture and tetanus ..	4
Smith-Petersen's pinning ..	4
Intramedullary nailing ..	6
Sprains ..	12
Fracture of neck of femur with delirium tremens ..	4
Paraplegia from spinal fracture and bed sores ..	6
Plastering ..	12
Osteomyelitis-acute ..	6
-chronic ..	8
Tuberculosis of bones ..	9
Pyogenic infection of joints ..	6
Gonorrhoeal arthritis ..	2
Tuberculosis of joints ..	8
Solitary and multiple exostosis ..	8
Synovial chondromatosis and malignant syno- vioma ..	3
Monostotic fibrous dysplasia ..	3
without fracture ..	3
with fracture ..	3
with operation ..	4
Osteogenic sarcoma ..	



Types	Number
Osteoclastoma	
without pathological fracture	4
with pathological fracture and pain	2
with bone grafting operation	4
Secondary cancers of bone	2
Thorn test	48

#### Control Cases :—

The count varied from 72 cells per c. mm. to 375. The counts in the control cases have been taken from normal healthy persons (46) free from diseases. There is good agreement in the count between this group and the pre-operative counts in the patients subjected to orthopaedic surgery. In the control cases, the counts have been repeated many times, at the same period on different days with consistent results.

#### Anaesthesia :—

Ether, or N<sub>2</sub>O-oxygen and ether have been used as anaesthetic agents for the operations. In many cases the eosinophil count starts falling even before anaesthesia is induced, and this is due to the psychological tension on the part of the patient which leads to increased corticoid secretion from the adrenal cortex. In others, eosinophil counts start falling during the induction of anaesthesia and by the time the operation is finished many patients show the level to be zero or a very low level is found. Nicholas *et. al.* (1953) could not find any lowering of eosinophil cells during anaesthesia. But this has been noticed by me and this is in full agreement with the observations made by Moore (1955).

#### Cases without post-operative complications :—

The total number of cases studied in this group is 65 ; of these 37 cases have been presented in the figure. There is no post-operative complications in these cases. The operations include sequestrectomy, osteotomy, bone grafting and bone plating for fractures, bone grafting for osteoclastoma and monostotic fibrous dysplasia, spinal fusion operation for caries spine, Naughton Dunn's operation, arthroplasty, arthrodesis, tenotomy, synovectomy, and amputation of limbs for osteogenic sarcoma etc.

The pre-operative counts varied between 72 and 375 but only very few patients (2) showed counts higher than 375.

#### Cases with post-operative complications :—

The total number of these cases is 22. The post-operative complications include operative wound infection, lung complications, abdominal distension with paralytic ileus. These patients had persistent eosinopenia till the clinical condition improved. Others show eosinophilia when the clinical condition is not good. These patients require careful watch and treatment.



### Cases with simple fracture :—

34 cases have been studied. The group includes fracture of femur, tibia and fibula, spine, humerus, or both bones of forearm. The count is either zero or very low count has been encountered in this group. The counts begin to rise on the 2nd day and by the 3rd day they are above hundred except in two cases where they are 93 and 86 cells per c. mm.

### Cases with multiple fractures :—

20 cases have been studied in this group. This includes fractures of the spine, femur, fibula, humerus and both bones of forearm in combinations. It has been found that low eosinophil counts persist upto the 6th or 7th post-traumatic day and then they gradually rise up indicating thereby that the stress in this group is more severe in comparison to the simple fracture group.

### Cases with compound fracture :—

28 cases have been studied in this group. The eosinopenia persists for about two weeks ; but it would be more correct to state that eosinopenia depends on the degree of infection in this group. For the first few days the eosinopenia is due to the stress of the fracture ; for the subsequent eosinopenia, the infection is responsible. The quicker the infection is combated, the quicker return from eosinopenia to eosinophilia occurs. Curiously enough, compound fracture with bullet injuries shows more prolonged eosinopenia.

### Cases with compound fractures with gas gangrene or tetanus :—

6 cases with gas gangrene and 4 with tetanus have been studied. Here also the eosinopenia persisted so long as the patients' clinical condition was bad.

### Smith Petersen's pinning operation :—

4 cases have been studied. The eosinopenia persisted for 3 to 4 days followed by eosinophilia.

### Intramedullary nailing operation for fracture of the shaft of the femur :—

6 cases have been studied. In these cases the eosinopenia persisted for about 3 weeks. This is in full agreement with the work of Nicholas and Wilson (1953) but differs from that of Moore *et. al.* (1955). I have used Henry's method of exposure for the midshaft of femur. Prolonged eosinopenia may be explained by the amount of wound produced during operation. There are other methods too for this operation where small incisions are made. Moreover the presence of a long foreign material in the intramedullary canal may alter the local tissue reaction, thus inciting the increased adrenocortical activity for a long time. I did not use any form of external immobilization after the operation.

### Sprains :—12 cases have been studied.

The eosinopenia is short lasting. It rebounds to eosinophilia when the pain diminishes after 2% procaine injection into the injured ligament.



### **Fracture of the neck of femur with delirium tremens :—**

4 cases have been studied. Eosinopenia persists so long as the patient's condition is bad. On the improvement of the general condition eosinopenia rebounds to eosinophilia.

### **Paraplegia from spinal fracture with bed sores :—**

6 cases—Eosinopenia persists for about 3 weeks. After that, though the wound is there, the eosinophils rise.

### **Plastering :—**

12 cases—Plastering for caries spine or tuberculosis of the knee or hip joints did not give rise to eosinopenia. Eosinopenia has been found only in the cases where there is lung complication or abdominal distension requiring removal of the plaster.

### **Osteomyelitis :—**

6 cases in the acute stage and 8 in the chronic stage have been studied. Eosinopenia in the acute stage persists for about 2 weeks or till the subperiosteal abscess is drained surgically. Then the eosinopenia rebounds to eosinophilia. In chronic osteomyelitis with sequestrum and sinuses no eosinopenia has been found. Eosinopenia occurs when sequestrectomy is done.

**Tuberculosis of bones (9 cases)**—No eosinopenia has been found.

### **Pyogenic infection of joints (6 cases) :—**

Eosinopenia persists till the joint is drained. When the patient's clinical condition improves, the eosinopenia rebounds to eosinophilia. In Gonorrhoeal arthritis (2 cases) the same feature is found.

### **Tuberculosis of Joints (Hip and Knee) (8 cases) :—**

Patients suffering from excessive pain in the 1st and 2nd stage of the disease show eosinopenia. When the pain diminishes and the joints are immobilised eosinophilia occurs.

### **Solitary and multiple exostosis (8 cases) :—**

There is no eosinopenia.

### **Synovial chondromatosis and malignant synovioma :—**

3 cases—no eosinopenia is found.

### **Monostotic fibrous dysplasia :—**

(a) Without fracture—3 cases—no eosinopenia.

(b) With pathological fracture—3 cases—eosinopenia for two to three days followed by eosinophilia on the immobilization of the limb.

(c) With multiple chip bone grafting operation—3 cases.

There was post-operative eosinopenia for two to three days followed by eosinophilia.

### **Osteogenic sarcoma—(4 cases)**

All these patients had excessive pain and showed eosinopenia. When the limbs are amputated eosinophilia occurs.



**Osteoclastoma :—**

(a) Without pathological fracture and without pain—4 cases. There was no eosinopenia.

(b) With pathological fracture and pain—2 cases—eosinopenia.

(c) Bone grafting operation—4 cases. No eosinopenia occurred in the pre-operative period. Post-operative eosinopenia was found for 3 to 5 days followed by eosinophilia.

**Secondary cancers of bone from prostatic carcinoma :—**

2 cases—These cases have been complicated by pathological fracture and they show eosinopenia.

**Thorn test with 25 mgm. of ACTH :—**

This test has been applied to pre-operative and post-operative patients and injured patients in the post-traumatic period. 48 patients have been investigated. In the pre-operative period majority of the patients shows fall of more than 50 per cent in the eosinophil count. In the post-operative or post-traumatic periods some patients show fall less than 50 per cent ; but when the dose has been doubled the fall is as usual :

**Conclusion :—**

Adrenocortical function assessed by eosinophil count has been studied in different types of bone and joint diseases and tumours, different types of fractures and their complications, in anaesthesia and in orthopaedic operations.

1. During anaesthesia (types used here have been mentioned before) or even in the pre-anaesthetic stage there is increased adrenocortical activity.

2. The duration of the eosinopenia in the post-operative period depends on the severity of the operation.

3. Post-operative cases with complications show prolonged eosinopenia. Some cases show eosinophilia.

4. In simple fracture, the eosinopenia may persist for 3 days.

5. In cases with multiple fractures, the stress is more severe and the eosinopenia persists for 6 or 7 days.

6. In compound fracture, eosinopenia is prolonged.

7. Cases with compound fracture and gas gangrene or tetanus show prolonged eosinopenia.

8. In Smith Petersen's pinning operation for fracture of neck of femur, the eosinopenia is for 3 to 4 days.

9. In intramedullary nailing operation, the eosinopenia is prolonged.

10. In sprains, the eosinopenia is short lasting.

11. In fracture of neck of femur complicated by delirium tremens, the eosinopenia persists till patient's clinical condition improves.

12. In paraplegia from spinal fracture with bed sores there is eosinopenia for about 3 weeks.

13. Plastering does not lead to eosinopenia.

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14. In chr. osteomyelitis there is no eosinopenia.  
In acute osteomyelitis there is eosinopenia.
15. In bone tuberculosis there is no eosinopenia.
16. In pyogenic infection of joints there is eosinopenia.
17. Tuberculosis of joints with pain shows eosinopenia.
18. In solitary and multiple exostosis, synovial chondromatosis and malignant synovioma there is no eosinopenia.
19. In monostotic fibrous dysplasia without fracture there is no eosinopenia. With fracture or operation there is eosinopenia.
20. Osteogenic sarcoma with excessive pain—eosinopenia.
21. Osteoclastoma—
  - (a) Without fracture—no eosinopenia.
  - (b) With fracture or operation—eosinopenia.
22. Secondary carcinoma of bone with pathological fracture—eosinopenia.



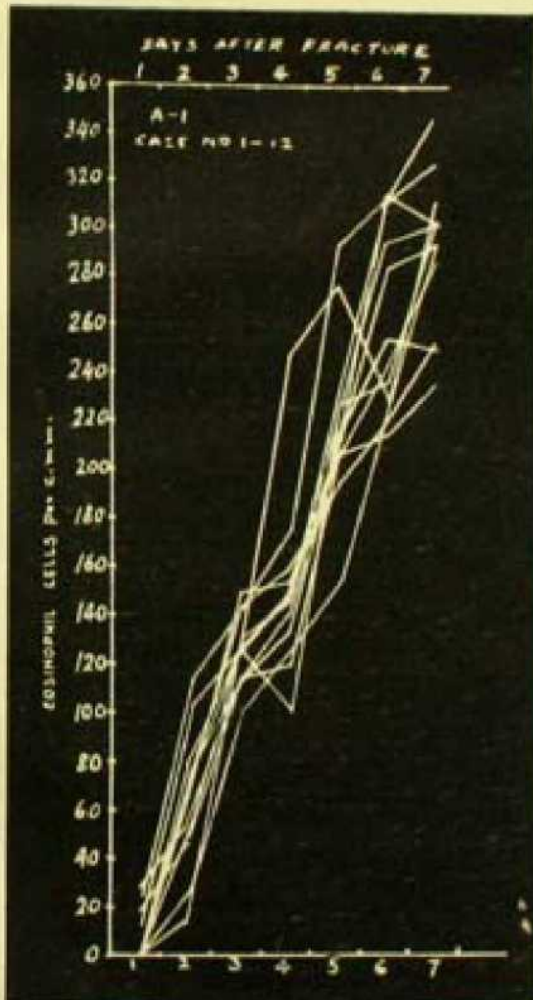


Fig. 1.—Eosinophil cell counts after simple fracture.

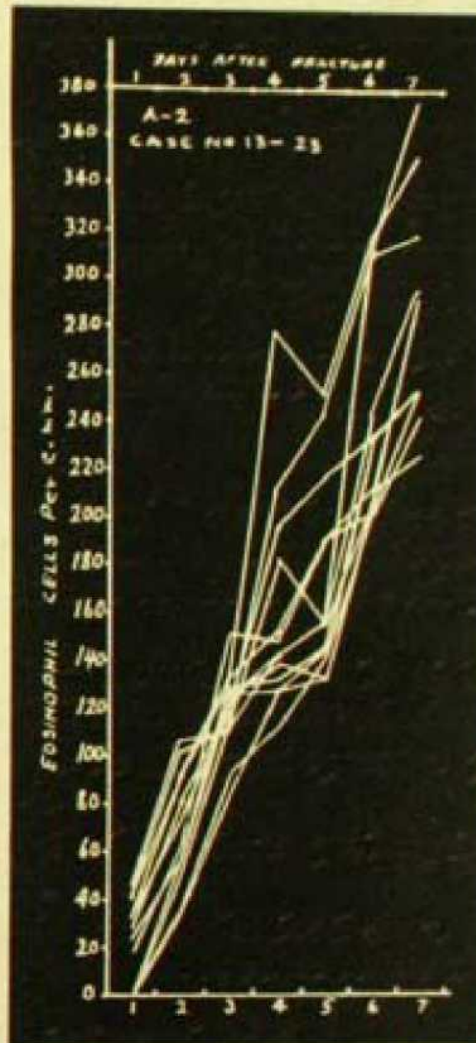


Fig 2.—Eosinophil cell counts after simple fracture.



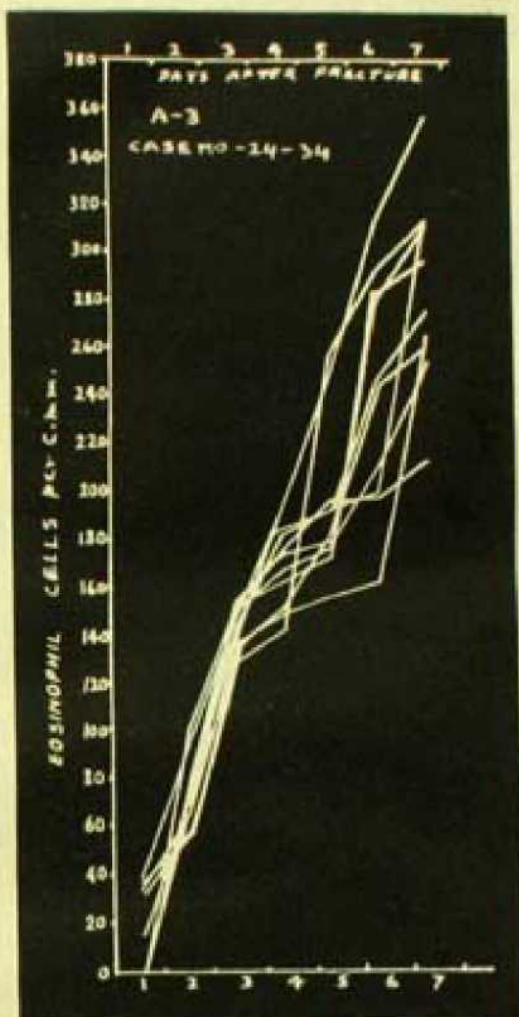


Fig. 3.—Eosinophil cell counts after simple fracture.

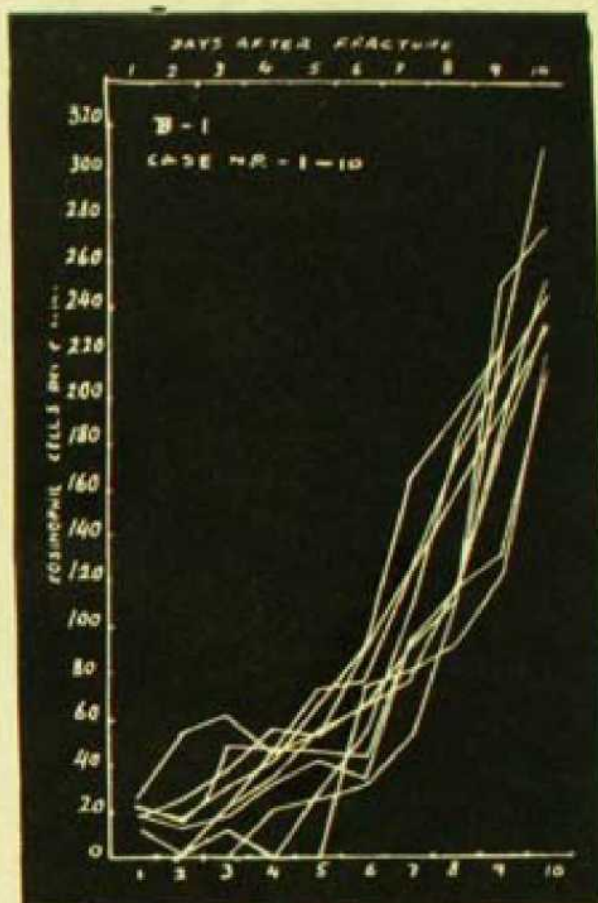


Fig. 4.—Eosinophil cell counts after multiple fracture (spinal+femur or humerus, both bones of leg or forearm fractures).

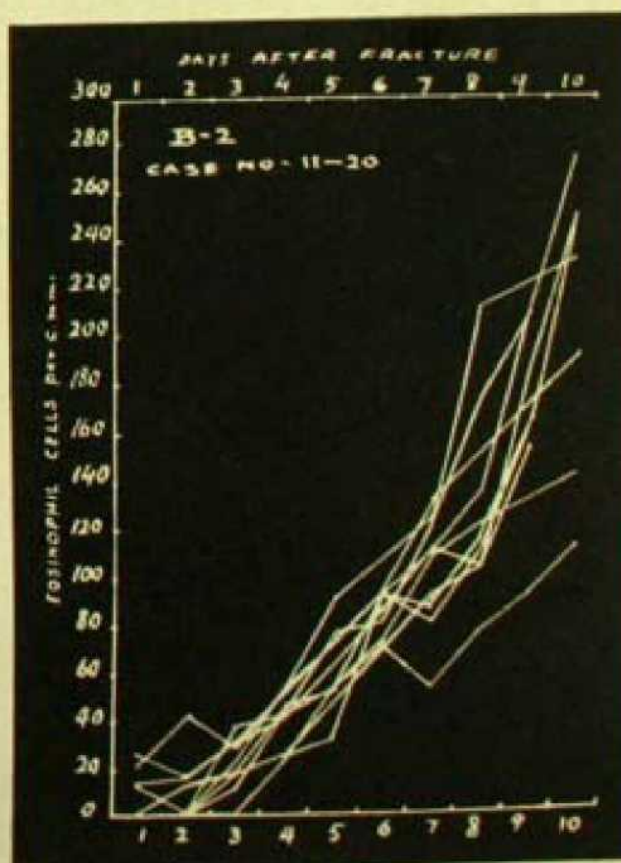


Fig. 5.—Eosinophil cell counts after multiple fracture (spinal+femur or humerus, both bones of leg or forearm fractures).



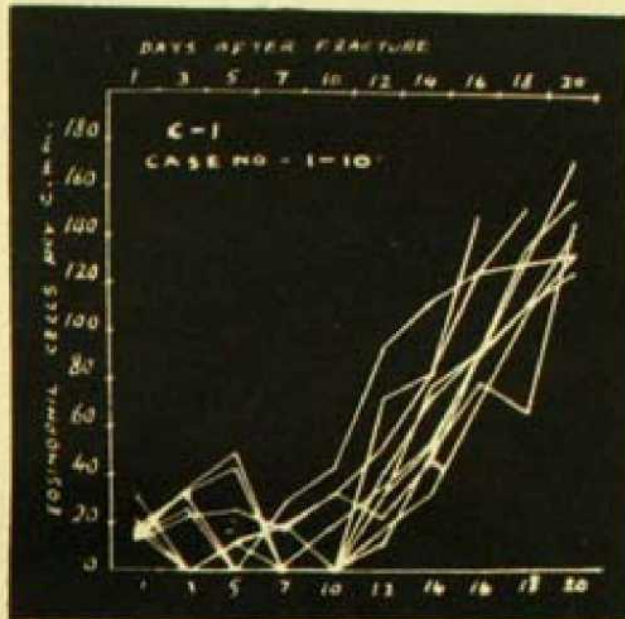


Fig. 6.—Eosinophil cell counts after compound fracture (includes femur, humerus, leg or forearm bones).

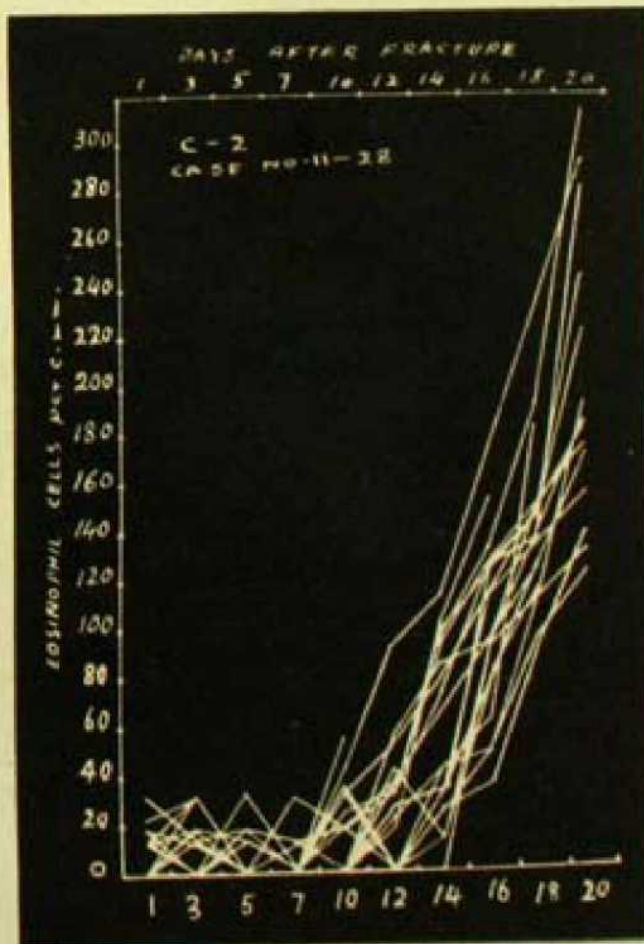


Fig. 7.—Eosinophil cell counts after compound fracture includes femur, humerus, leg or forearm bones).



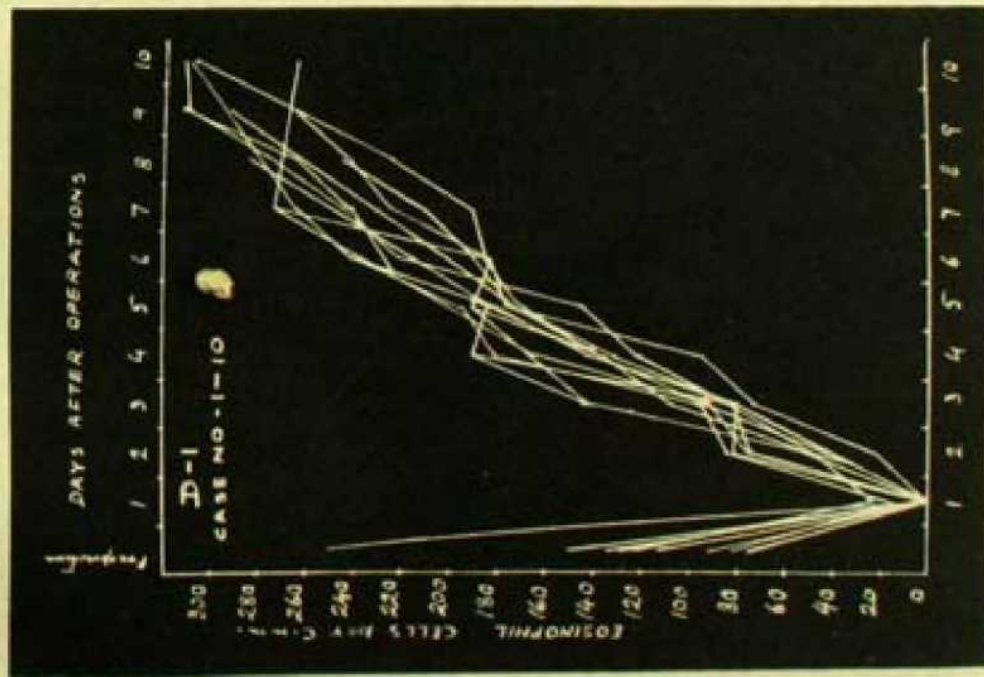


Fig. 8.—Changes in eosinophil cell counts after operations.

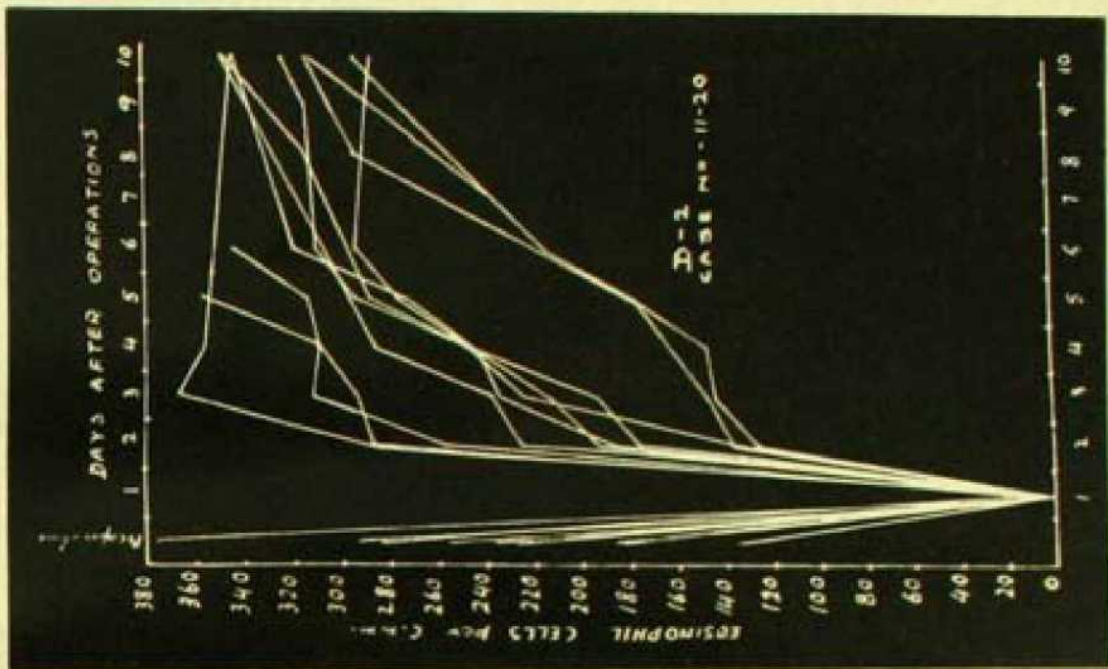


Fig. 9.—Changes in eosinophil cell counts after operations.



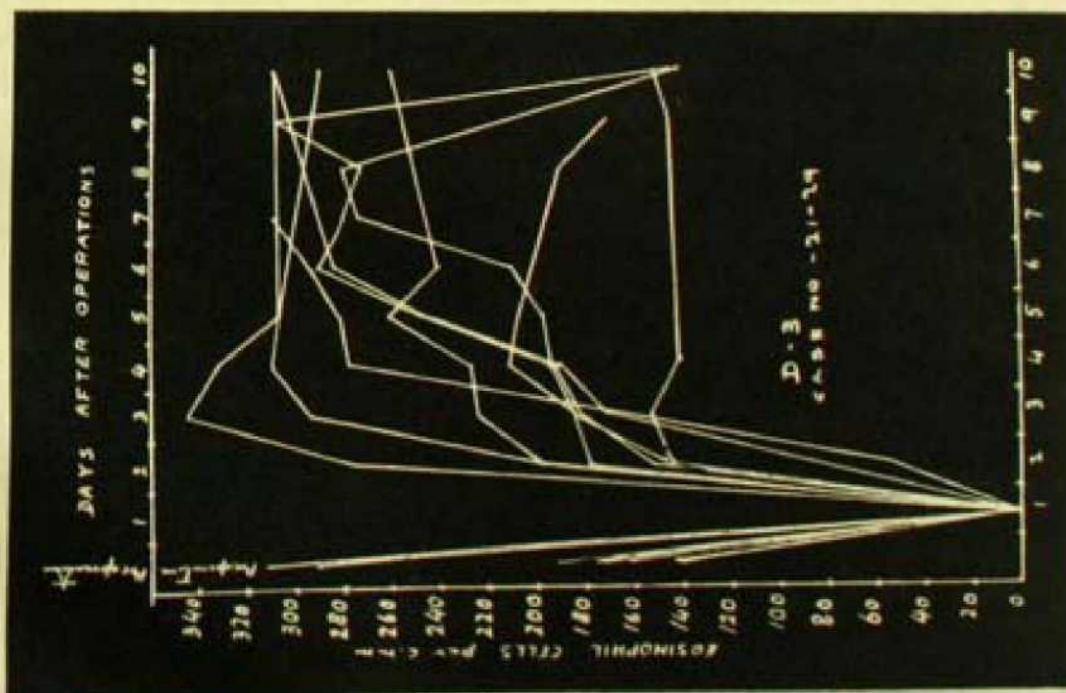


Fig. 10.—Changes in eosinophil cell counts after operations.

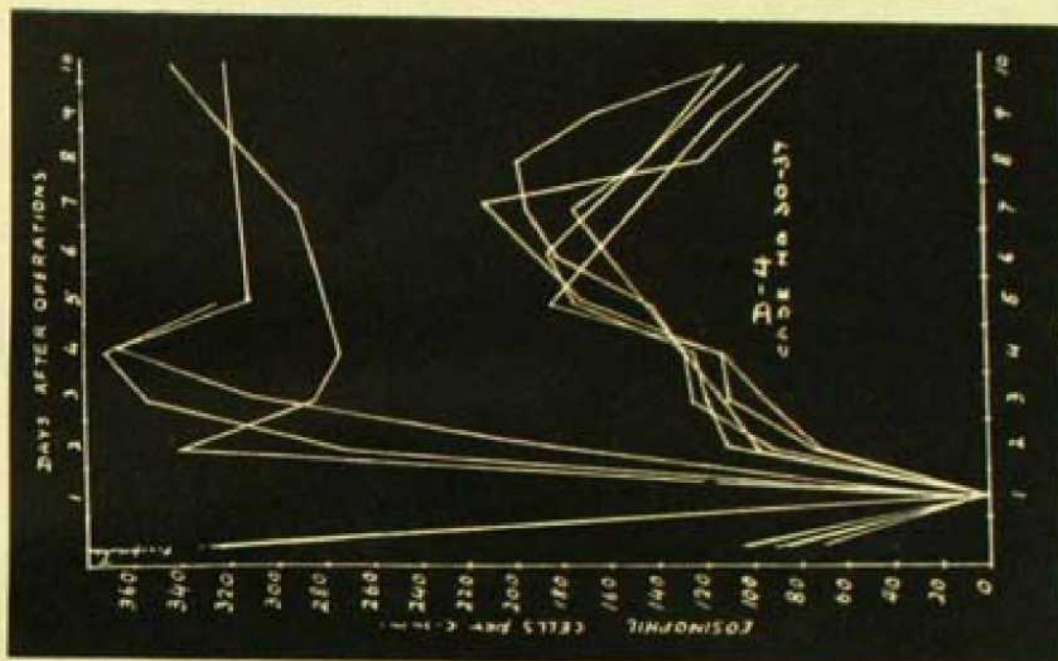


Fig. 11.—Changes in eosinophil cell counts after operations.



## CHAPTER II

### ADRENOCORTICAL STUDY IN MINOR TRAUMA, FRACTURE, BURN AND CLOSED LOOP INTESTINAL OBSTRUCTION IN NORMAL AND DENERVATED DOGS

Selye (1937) showed that there was adrenal gland enlargement after various types of stress. Torrance (1940), Sayers *et. al.* (1944, 1945), Fortier *et. al.* (1950) found depletion of adrenal ascorbic acid and cholesterol after stress. Trauma leads to fall in adrenal ascorbic acid content in various species (Stolfi, 1936; Long, 1947; Selye and Stone, 1950). Popjak (1944) found diminished neutral fat, free and ester cholesterol content of the adrenals after tourniquet shock in rats.

Regarding the mediator which would stimulate the secretion of ACTH during stress, Selye (1950) has said, "This intensive search for a mediator—which would be produced by injured cells and after having reached the pituitary would induce it to produce ACTH—may be doomed to failure because perhaps no single substance is responsible for this effect. It is generally agreed that a great variety of stressors eventually act through a common pathway, namely the anterior lobe. At present we have no reason to believe that the pathway is common at a higher level. It is quite possible that any derangement in the biochemical constitution of the blood (toxic substances, hormones, deficiency of certain vitally important metabolites, etc.) as well as nervous stimuli reaching the anterior lobe cells directly through nerve fibres, may liberate ACTH from some labile combination in the cell and stimulate its increased production." In 1954 and 1956, Selye discussed about the pathways for the stress message.

The somatic and sympathetic afferents are important for the stress response (Hume, 1952). He has shown that operative trauma to a denervated extremity in the dog did not show eosinopenia, but very severe burn trauma gave rise to slight eosinopenia. Gordon (1950) evaluated the importance of afferent nerves in the adrenocortical response to trauma. Adrenocortical response to trauma of lesser magnitude could be blocked by neurectomy but denervation had no effect on the adrenal ascorbic acid discharge after a more severe scald. He did only femoral and sciatic neurectomy, but he did not remove the influence of the sympathetic afferents. Porter (1954) observed that excitation of the sciatic nerve in spinal monkeys produced an average eosinopenia of  $-54\%$  ( $\pm 10.6$ ). Similar experiments in monkeys with intact spinal cord produced eosinopenia of  $-63\%$  ( $\pm 8.8$ ). In dogs with cervical cord section, Anderson (1954) did not find any stimulation of the ACTH—adrenal cortical system after laparotomy and hemidecortication by measuring formaldehydogenic substances in the urine. The reason of this finding is that due to cord section there is an interruption of the nervous path from the hypothalamus to the adrenal medulla by the splanchnics and thus an important chain for the ACTH-adrenocortical mechanism is missing. Wilson *et. al.* (1956) found that the



stress of laparotomy in the spinal dogs (C 7) gave rise to adrenocortical stimulation. Transection at the level of the midbrain blocked the adrenocortical response to laparotomy. Roche *et. al.* (1950) found delay in the eosinopenia after surgical operations carried on under spinal anaesthesia. Long (1950) could not find eosinopenia in spinal rats in response to laparotomy and intestinal manipulations at 1 hour. However at 4 hours the eosinopenia occurred.

McDermott *et. al.* (1950) and Long (1952) suggested that after stress the following events occurred to give rise to the discharge of ACTH. First of all the hypothalamus got stimulated and it transmitted the impulses through the brain stem to the adrenal medulla to give rise to the secretion of epinephrine. The liberated epinephrine reached the anterior pituitary to stimulate the ACTH secretion. Long (1952) also said that this was not the only mechanism but the purely humoral system came into action slowly and might not be required at all if the stress was of short duration.

Sayers (1957) studied the neural mechanisms for regulation of ACTH release from the adenohypophysis. In a normal animal, the rate of release of ACTH is balanced by the inhibitory action of corticosteroids and excitation caused by the neural mechanism. The pituitary response to some noxious stimuli is biphasic—an "excitatory" phase, in which there is rapid and marked increase of blood ACTH and the response in this phase to ether and to pitressin can be abolished by high pontine section. Epinephrine and acid extracts of ventral hypothalamus raise blood ACTH level in adrenalectomized decerebrate rats and these two agents seem to act rostral to the pons. The excitatory phase is followed by a "regressive" phase during which the level of ACTH in the blood comes down to the previous level inspite of continuous action of the noxious agent. The cause of the regressive phase may be (a) tolerance of the animal to the noxious agents or (b) an element of the neural mechanism which is engaged in the increase in pituitary ACTH activity, may show adaptation.

Royce and Sayers (1958) employed the exogenous test for ACTH release. Here the analysis of blood ACTH in a recipient hypophysectomized rat shows the rapid blood ACTH changes after the application of a stimulus. The endogenous test in the stimulated animal by noting the adrenal ascorbic acid depletion due to the release of ACTH does not show these dynamic changes. After a painful stimulus there is a marked increase in blood ACTH within a few minutes. During 30 minutes of intermittent painful stimulation there is elevated blood ACTH level. Ether showed a biphasic response. It first excited and then inhibited ACTH release. Pentobarbital showed a depressant action. The excitatory phase of ether can be blocked by decerebration and by the destruction of the median eminence. The excitatory action of epinephrine is seen in the decerebrate rats but not in the rats with lesion of the median eminence. The inhibitory action of ether and pentobarbital on ACTH release is due to the involvement of the multisynaptic conduction system (the brainstem reticular system). A tonic action on the pituitary is exerted by stimuli in everyday existence such as light, sound and touch and there is a low rate of release of ACTH.



From the above review the importance of the afferent nerves in the adrenocortical response after stress can be understood. In the present investigation, the study of the adrenocortical activity is based on the measurement of 17-hydroxycorticosteroids in the adrenal venous blood after different types of stressing procedure on :—

- (a) normal dogs.
- (b) dogs with denervation of the hind limb.
- (c) dogs with denervation of the hind limb and application of compression bandage after fracture.
- (d) midbrain sectioned dogs.

The intention of this study is to find out the role of the afferent nerves in adrenocortical activation during various types of stress and also to study the response in midbrain sectioned dogs.

#### Materials and methods :—

(1) Male dogs of 10—15 kg. in weight have been used. The daily diet has been kept constant. Water was freely available.

(2) Cannulation of the right adrenal vein for the collection of adrenal venous blood was done after the method of Hume and Nelson (1955). Adrenal venous blood samples have been collected in heparinized tubes. The dogs were not heparinized. After immediate centrifuging, the plasma was preserved in cold for the determination of 17-hydroxycorticosteroids after the method of Silber and Porter (1954).

(3) Normal dogs, partially sympathectomized dogs, dogs with denervated limb (by sectioning the Lumbar and Sacral plexus of nerves) and midbrain sectioned dogs have been used.

#### (4) Types of stress :—

- (a) Fracture of femur.
- (b) Burn trauma by immersing the hind limb in boiling water for two minutes.
- (c) Minor surgery by simply incising the skin and fasciae of the hind limb for three inches, separation of muscles, exposure of bone and then closure of the wound.
- (d) Intestinal obstruction—the first portion of the jejunum, 15 centimeters from the ligament of Treitz was obstructed after the technique of Stone, Bernheim and Whipple (1912). 10 to 12 centimeter portion of the gut was resected and the ends of this portion were inverted to form a closed loop. Then the continuity of the gastrointestinal tract has been achieved by end to end anastomosis.  
Denervation of the obstructed loop was done by dissecting and keeping the central vessels intact and sectioning all other structures in the mesentery. The vessels were painted with



20 per cent phenol. Denervation was also produced by infiltrating the mesenteric pedicle with 95 per cent alcohol—(Antonovic *et al.*, 1941).

### Results :—

During the adrenal vein cannulation in 20 dogs, the 17-hydroxycorticosteroid output varied from 16.9 to 22.4 gamma per minute with an average of 19.5 for all twenty dogs. This high value came down to low level on the 1st and 2nd postcannulation day. After the stress of fracture the 17-hydroxycorticosteroid output varied from 20.4 to 31.2 gamma per minute with an average of 26.9 for all twenty dogs. In 9 dogs with denervation (both sympathetic and somatic) of the extremity, the corticosteroid output was from 14.4 to 19.4 gamma per minute with an average of 17.3 for all the dogs during cannulation. After fracture the values ranged from 6.4 to 12.6 gamma per minute with an average of 9. In 5 dogs with fracture and compression bandage applied to the denervated limb, there was no increase in 17-hydroxycorticosteroid output.

Stressing effect of fracture has been compared to the effects of minor surgery, burn trauma and intestinal obstruction.

In 5 dogs, minor surgery to the innervated limb showed more increased 17-hydroxycorticosteroid output per minute than that is found in dogs with denervated limbs. In such dogs, though the corticosteroid output during adrenal vein cannulation and denervation was 14.3 gamma per minute (average of 5 dogs), still after minor surgery it is 2 gamma per minute which approximates well towards the prestress value of 1.9 gamma per minute.

In burn trauma applied to the neurectomized limbs in 5 dogs, the 17-hydroxycorticosteroid output ranged from 18.6 to 28.3 gamma per minute with an average of 24.1 for 5 dogs. This value is higher than that found during minor surgery or after fracture in similar animals. Burn injury applied to non-denervated limbs showed more increased adrenocortical activity. (35.1 gamma per minute, average.)

In 5 dogs with intestinal obstruction and without denervation of the obstructed loop, the survival period was 64 hours (average) with higher 17-hydroxycorticosteroid output per minute (27.3 gamma—average). The obstructed loops of the animals in this group had perforation and the animals suffered from peritonitis. In five animals with intestinal obstruction and with denervation of the obstructed loop, the survival period was more prolonged. The 17-hydroxycorticosteroid output for three days after operation was high.

On the 1st post-operative day ..	15.8 gamma/min.—average of 5 dogs
On the 2nd " " " " ..	17.3 gamma/min. " "
On the 3rd " " " " ..	18.2 gamma/min. " "

This condition varies from other operation in that in the latter condition the corticosteroid output comes down to low values within the second post-operative day. Whereas, studies on the third day and even subsequently showed increased corticosteroid output in dogs with intestinal obstruction.



Stresses such as fracture, burn and intestinal obstruction have been studied in midbrain sectioned dogs. Three dogs with each type of stress have been studied. In these animals the adrenal venous 17-hydroxycorticosteroid output was at a lower level. With fracture, the increase of the 17-hydroxycorticosteroid output per minute as compared to prestress values for three dogs is 4.3, 3.2 and 5.2 with an average of 3.9. For burn, the increase is 8.2, 7.1, 4.3 with an average of 6.5. For intestinal obstruction it is 6.4, 8.3 and 7.4 with an average of 7.3. It is found that in animal with transection at the level of midbrain, the rise in 17-hydroxycorticosteroid output after application of stress is much less compared to other groups. The rise is minimum in fracture and maximum in intestinal obstruction.

### Effect of anaesthesia and adrenal vein cannulation and fracture on 17-hydroxycorticosteroid output per minute

Dog No.	Weight in Kg.	17-hydroxycorticosteroid output gamma/min. during anaesthesia and adrenal vein cannulation.	17-hydroxycorticosteroid output gamma/min. on 1st post-operative day.	17-hydroxycorticosteroid output gamma/min. on 2nd post-operative day.	17-hydroxycorticosteroid output gamma/min. after fracture.
1	10	18.2	4	2.3	30.1
2	10.5	17.4	2.4	2.1	28.2
3	11	19	3.1	1.7	21.9
4	12	18.4	5.4	2.4	26.4
5	10.5	17.6	0	1.6	25.9
6	14	16.9	4.1	2.8	22.8
7	13	19.2	2.6	3.2	28.4
8	12	20.1	5.1	2.7	26.1
9	12.5	18.7	4.9	1.6	25.4
10	15	17.9	3.3	0	30.8
11	14.5	19.4	5.3	0	29.9
12	14	21.4	4.2	3.2	30.2
13	13	20.7	3.9	4.2	28.2
14	12.5	22.4	2.6	3.9	27.6
15	10	18.9	1.7	2.1	26.9
16	10.5	22.2	4.3	1.7	20.4
17	11	21.4	4.4	1.5	22.8
18	12.5	21.08	3.8	1.2	28.6
19	13.5	18.9	2.3	1.4	27.5
20	15	19.1	2.5	1.6	31.2
Average		19.5	3.4	2	26.9



### 17-Hydroxycorticosteroid output in sympathectomized and neurectomized dogs with fracture

Dog No.	Wt. in Kg.	During Pentobarbital anaesthesia and cannulation of the right adrenal vein.	1st postoperative day.	2nd postoperative day.	Fracture.
17-Hydroxycorticosteroid output gamma/minute.					
21	15	15	8.1	2.3	10.1
22	12.5	18.1	5.3	2.6	6.4
23	12	19.4	2.6	1.6	8.6
24	13	16.2	4.2	2.9	9.8
25	14.5	14.4	3.2	2.7	6.5
26	15	18.6	2.1	1.9	7.6
27	14	17.8	1.8	2.1	12.6
28	13	15.6	1.7	1.9	10.8
29	10	16.1	1.5	1.4	8.9
Average		17.3	3.3	2.1	9

### 17-Hydroxycorticosteroid output in sympathectomized and neurectomized dogs with fracture and compression bandage

Dog No.	Wt. in Kg.	Neurectomy and adrenal vein cannulation.	Before fracture.	After fracture and compression bandage.
17-Hydroxycorticosteroid output gamma/minute				
30	13.5	16.4	1.8	1.9
31	12	10.2	2.1	2.3
32	10	18.3	1.6	1.4
33	10.5	20.1	1.4	1.3
34	14	15.3	1.2	1.6
Average		16	1.6	1.7

### 17-Hydroxycorticosteroid output in sympathectomized and neurectomized dogs with minor surgery

Dog No.	Wt. in Kg.	Neurectomy and adrenal vein cannulation.	Before minor surgery.	After minor surgery.
17-Hydroxycorticosteroid output gamma/minute				
35	14.5	14.2	2.4	2.2
36	12	12.3	2.1	1.8
37	13.5	16.4	1.8	2.1
38	14	13.2	2.2	2.3
39	15	15.4	1.2	1.8
Average		14.3	1.9	2



### 17-Hydroxycorticosteroid output in sympathectomized and neurectomized dogs with burn

Dog No.	Wt. in Kg.	Neurectomy and adrenal vein cannulation.	Before burn.	After burn.
17-Hydroxycorticosteroid output gamma/minute				
40	15	16.2	1.8	18.6
41	12	15.3	1.3	20.2
42	13	14.8	2.2	27.3
43	14.5	17.6	2.6	26.4
44	11	14.2	1.3	28.3
Average		15.6	1.8	24.1

### 17-Hydroxycorticosteroid output in dogs with denervated closed loop intestinal obstruction

Dog No.	Wt. in Kg.	Intestinal obstruction.	1st post-operative day.	2nd post-operative day.	3rd post-operative day.
17-Hydroxycorticosteroid output gamma/minute					
45	14	18.6	14.4	15.8	14.8
46	13	20.2	18.6	16.9	20.2
47	12	22.2	18.3	19.4	19.1
48	10.5	18.9	17.5	18.2	18.6
49	11.5	23.2	10.2	16.5	18.6
Average		20.6	15.8	17.3	18.2

### 17-Hydroxycorticosteroid output in midbrain sectioned dogs with fracture, burn and intestinal obstruction

Dog No.	Wt. in Kg.	Type of stress.	Rise of 17-Hydroxycorticosteroid output gamma/minute.	Average.
50	10	Fracture	4.3	3.9
51	10.5	Fracture	3.2	
52	14	Fracture	5.2	
53	15	Burn	8.2	6.5
54	12.5	Burn	7.1	
55	11	Burn	4.3	
56	11.5	Intestinal obstruction	6.4	7.3
57	10	Intestinal obstruction	8.3	
58	12	Intestinal obstruction	7.4	



### Discussion :—

Anaesthesia and cannulation of the right adrenal vein gave rise to increased corticoid output from the adrenal. The high value came down to normal within first and second post-operative day. This observation agreed with that of Hume and Nelson (1955). After fracture of femur in such dogs there is increased 17-Hydroxycorticosteroid output per minute but the values are higher than those found during anaesthesia and cannulation of the adrenal vein. When the limb is denervated, trauma of fracture cannot elicit the adrenocortical response to a degree which is comparable to that found during fracture of an innervated limb. The response is less. This is because of absence of the afferent path which is required for the increased adrenocortical activity according to Long's hypothesis. But still the response that is achieved in this group is higher than the basal output of corticoids. This may be due to the absorption of chemicals liberated at the fracture site and their action on the pituitary or adrenals. This is also proved by experiments in which absorption of chemicals is kept in abeyance by means of a compression bandage made up of rubber and applied to a denervated extremity after fracture. It is found that there is no increase in 17-Hydroxycorticosteroid output per minute. Importance of afferent nerves for increased adrenocortical activity during stress has been observed by Long (1950), Gordon (1950), Roche *et al.* (1950), Hume (1952), Anderson (1954). However, Hume (1952) found eosinopenia after more severe burn trauma applied to a denervated limb. Porter (1954) observed eosinopenia after excitation of sciatic nerve in spinal monkeys.

The adrenocortical activity found in fracture has been compared to that during minor surgery, burn trauma and intestinal obstruction. Minor surgery applied to a denervated limb did not show appreciable increase in the corticoid output. Similar trauma applied to an innervated limb showed higher values but still they are lower than those found during anaesthesia and adrenal vein cannulation. This shows that during minor surgery, afferent nerves are very important for eliciting increased adrenocortical activity.

The study of the burn trauma has been included as in this condition there is much increased adrenocortical activity. Increased chemocorticoids in the urine of burn patients have been found by me (Roy, 1953). Evans and Butterfield (1951), Wight and her associates (1953) and Hardy (1955) have found increased adrenocortical activity after burn. Direct measurement of bio and chemocorticoids from adrenal venous blood after burn injury shows increased adrenocortical activity (Roy, 1953). In the present investigation, burn trauma to the neurectomized limbs of dogs leads to increased 17-Hydroxycorticosteroid output per minute from the adrenal. The magnitude of response is greater in burn injury to the innervated limb and also it is greater than the response observed after fracture to the denervated limb. Hume (1952) observed eosinopenia of lesser magnitude after burn trauma to the denervated limb of a dog. Direct measurement of the corticoids in the adrenal venous blood, however,



shows much increased activity. This increased activity even after denervation may be due to the release of chemicals from the burned site into the general circulation through which they may act on the pituitary or adrenals. This may be explained also by feed-back mechanism as described by Sayers.

Neurogenic factor is very important in the production of symptoms in closed-loop intestinal obstruction and therefore this type of intestinal obstruction has been studied in the present investigation. Burget and his associates (1930), Herrin and Meek (1933), Taylor and his associates (1933), and Antoncic and Lawson (1941) observed lesser symptoms and longer survival periods in dogs with denervated closed intestinal loops. Antoncic and Lawson (1941) found the survival period to be 36 hours to 7 days in control dogs whereas dogs with denervated loops survived 3 weeks to 4 months. This shows the importance of the nervous factor in the production of symptoms and the influence over the survival period in closed-loop intestinal obstruction. Increased adrenocortical activity in acute intestinal obstruction has been found by me (Roy, 1950). In the present investigation, it has been found that denervation of the closed intestinal loop definitely increased the survival period of dogs. 17-Hydroxycorticosteroid output is high even in the absence of the nerves and this may be due to the absorption of chemicals liberated at the site of obstruction. The value is higher in closed-loop obstruction without denervation (27.3 gamma per minute—average) and the survival period is much lower than that observed in animals with denervated closed intestinal loops.

Sayers (1957) observed that the section through the brain stem at the upper level of pons abolished the excitatory phase of the pituitary response to ether and to pitressin. Transection at the level of midbrain blocked the adrenocortical response to laparotomy in dogs (Wilson *et al.*, 1956). Newman *et al.* (1958) reported that secretion rates of aldosterone or hydrocortisone in cats were not altered after transection of the midbrain reticular formation but significant reduction was noted after destruction of the ventral diencephalon. By lesions in the reticular formation of the midbrain and caudal diencephalon there was greater reduction in aldosterone levels and depression of the secretion of hydrocortisone. Lesions extending caudally into the rostral half of the pons resulted in significant increase in the secretion of aldosterone without affecting hydrocortisone secretion. In the present study, stresses such as fracture, burn and intestinal obstruction did not give rise to much increase in the 17-Hydroxycorticosteroid output per minute in the midbrain sectioned dogs ; but there was slight increase, the maximum with intestinal obstruction and the minimum with fracture.

### Conclusion :—

- (1) Anaesthesia and adrenal vein cannulation lead to increased 17-hydroxycorticosteroid output. This observation corroborates that of Hume and Nelson (1955).



- (2) In minor surgery, fracture, burn and intestinal obstruction, there is increased adrenal venous corticoid output. With denervation, minor surgery does not lead to appreciable rise in the output of corticoid, but rise in 17-hydroxycorticosteroid output is found in fracture, burn and intestinal obstruction after denervation. Fracture in a neurectomized limb with compression bandage applied does not show increase in 17-hydroxycorticosteroid output. Survival period of the animal is increased with denervation of the closed intestinal loop.
- (3) In midbrain sectioned dogs, the increase in the 17-hydroxycorticosteroid output after fracture, burn and closed-loop intestinal obstruction is not much and this suggests a control of the adeno-hypophysis by the midbrain mediated through the hypothalamus.



## CHAPTER III

### SAYERS' PERIPHERAL HUMORAL THEORY

Sayers and Sayers (1948) and Sayers (1950) stated that the concentration of the cortical hormone in the blood could regulate the ACTH secretion. Thus the pituitary-adrenal system could work in the same way as the pituitary-gonad and the pituitary-thyroid systems. During optimal environmental conditions, the peripheral tissue cells utilized small quantities of cortical hormone and thus the concentration of the cortical hormone in the blood was such that it could keep the anterior pituitary in check and minimum amount of ACTH was discharged. During stress the demand of the tissues for the cortical hormone was great and so the cortical hormone titer in the blood was low and this could lead to the increase in ACTH secretion.

Ingle and Kendall (1937) and Ingle *et al.* (1938) found adrenal atrophy after chronic treatment with adrenocortical extract.

Similar atrophy was produced by DCA (Selye, 1940) and cortisone (Lewis *et al.*, 1950). In man, symptoms of adrenal insufficiency could be produced after cessation of chronic treatment with DCA or cortisone (Zierler and Lilienthal, 1948 ; Forsham *et al.*, 1950 ; Sprague, 1951). Atrophy after cortical hormone administration was shown also by (del Castillo and Rapela, 1945 ; Sarason, 1943 ; Villela, 1941). Adrenal Hypertrophy subsequent to a large number of stressing agents was inhibited by DCA. (Selye and Dosne, 1942 ; Beznak and Korenyi, 1941 ; Hoen *et al.*, 1930 ; Albert and Selye, 1942 ; Woodbury *et al.* 1950.)

Ingle *et al.* (1938) suggested that the secretory activity of the anterior pituitary was guided by either an increase or decrease of cortin. Ingle (1937) could prevent the adrenal hypertrophy in the rat during exercise by treatment with cortical extract. Sayers and Sayers (1948) could block the increase in the pituitary adrenocorticotrophic activity in animals subjected to cold, heat, histamine, epinephrine and typhoid organisms by treatment with cortical extract or crystalline cortical steroids. There was quantitative relationships in this mechanism. "The degree of inhibition of pituitary adrenocorticotrophic activity is proportional to the amount of administered cortical hormone. The greater the degree of stress the greater is the amount of cortical hormone required to inhibit pituitary adrenocorticotrophic activity.... These data may be interpreted to mean that the peripheral tissues utilize cortical hormones at an accelerated rate during stress." Of the crystalline steroids, the most potent pituitary inhibitory substances were 17-hydroxy-corticosterone and 17-hydroxy-11-dehydro-corticosterone. Long (1947a, 1947b) found blocking of adrenal ascorbic acid depletion after cold or unilateral adrenalectomy or epinephrine by adrenocortical extract. Gellhorn and Frank (1948) found inhibition of lymphocytopenic action of epinephrine by adrenocortical extract. Inhibitory action of the adrenocortical extracts seemed to be on the anterior pituitary or the hypothalamus.



According to Long (1952) and Sayers (1950), exogenously administered corticoids could not inhibit the ACTH release completely after strong stimulation. Sayers (1950) said, "We have not been able to block completely the discharge of ACTH which follows severe stress. The mechanism may apply only to mild and moderate degrees of stress. In severe stress, anoxia or tissue toxins may have a direct action on the adenohypophysis. Likewise very large quantities of epinephrine may have a direct action on the adenohypophysis." Previous treatment with cortical steroids could not block the increased cortical activity in animals subjected to severe stress. (Sayers and Sayers, 1947; Swift *et al.*, 1948; Pinchot *et al.*, 1949; Gershberg *et al.*, 1950; Moya and Selye, 1948). Fortier *et al.* (1951) found that cortisone in the free or acetylated form, at dose levels of 0.5, 5, or 25 mg./100 gm. in aqueous or oily suspensions, administered subcutaneously or intraperitoneally decreased the adrenal ascorbic acid depletion after various types of severe stress including cold, histamine, audiogenic stimuli and fracture of both the hind legs of rats. "These results show that ACTH release in response to stress is compatible with a high level of circulating corticoids and that hypocorticoidism is not the sole responsible agent for the activation of the adrenocorticotrophic function."

Harris and Fortier (1954) said, "The most direct proof that the feed back mechanism is not necessary for the response to stress was offered by Sayers himself who reported that two minutes after scalding, exsanguination or ether anaesthesia, a significant increase could be detected in blood ACTH content of bilaterally adrenalectomized rats, and consequently in complete absence of circulating adrenal hormones." Moreover, Selye (1950) thought of some mechanism other than Sayers' hypothesis, otherwise it would be difficult to explain the increased ACTH discharge even after administration of large amounts of exogenous corticoids.

Fleming and Farrell (1956) said, "Although a reciprocal relationship may exist between adrenal aldosterone output and the release of an aldosterone-stimulating tropic hormone, these findings suggest that it is not the only or the dominant mechanism for regulation of aldosterone secretion."

#### Excess Removal of Corticoids by the Traumatized Area :—

This has not been shown to occur. Hume (1952) experimented on bilaterally adrenalectomized dog maintained on DCA pellet. A small dose of compound F was found out which when injected intra-arterially showed just significant eosinopenia. The same dose of compound F was now injected intraarterially into the traumatized limb of the dog. If according to Sayers' thesis traumatized tissues removed compound F, less of this substance would come into the circulation and would show less eosinopenia; but this did not happen. With a dose of compound F, the same amount of eosinopenia occurred in the adrenalectomized dog with or without trauma. This showed that the traumatized area did not use compound F directly from the blood stream.



Thorn *et al.* (1953) showed that Addisonian patients with or without surgical trauma excreted the same amount of 17-hydroxycorticoids in the urine when they were injected with a given dose of cortisone.

Cowie *et al.* (1954) could not demonstrate increased tissue utilization of corticoids by the injured adrenalectomized dog with graded doses of hydrocortisone. They said, "These and other data throw doubt on the hypothesis that increased corticoid utilization is responsible for the pituitary release of ACTH in response to operative trauma."

### The Rapidity of ACTH Response after Stress :—

Gray and Munson (1951) found that within 10 seconds after histamine injection there was ACTH release. Hume (1953) thought that this period would be too less for the tissue cells to take up corticoids from the blood stream and thus less of the corticoids would reach the anterior pituitary to stimulate ACTH secretion.

### Blood and Urinary Corticoids after Trauma in Man :—

Weichselbaum *et al.* (1953) found increased blood corticoids after trauma. Franksson *et al.* (1954) also found rise in 17-hydroxycorticoids in the peripheral blood of human after trauma. Similarly increased urinary excretion of corticoids was found by many. (These have been reviewed previously.) According to Sayers' thesis diminution in the blood corticoids was expected, otherwise stimulation of ACTH secretion would not occur. But this does not happen actually in stressed conditions.

Sayers (1951) suggested the possibility of a single neurohumor sensitizing the animal's pituitary to the various target gland hormones. As for example :—

(1) Neurohumor+change in thyroid hormone titer in body fluids :—modifies the rate of discharge of TSH.

(2) Neurohumor+change in cortical hormone titer :—modifies secretion of ACTH.

From the above review it is found that increased ACTH discharge in response to stress cannot solely be explained by Sayer's thesis. But still this hypothesis can explain the following facts :—

- (a) adrenal regeneration after enucleation of adrenal glands.
- (b) Compensatory hypertrophy of the remaining adrenal after unilateral adrenalectomy.
- (c) Hypertrophy of both the adrenals of the intact animal after bilateral adrenalectomy of the parabiotic twin.
- (d) Compensatory hypertrophy can be prevented by hypophysectomy.
- (e) Increased ACTH discharge in response to stress from anterior pituitary tissue transplanted in the anterior chamber of the eye.
- (f) Increased ACTH content of the blood of the adrenalectomized rats.



(g) Increased ACTH content in the blood of Addisonians or untreated patients with adrenocortical insufficiency.

Though there are many objections to this theory still this may be thought to work in the basal non-stressed conditions of the body and also one of the many mechanisms working during stress to increase ACTH secretion. Ulrich and Long (1956) could not find increased rate of utilization of adrenocortical hormones by the rat during exercise and after epinephrine injection ; but they have very rightly stated, "The term **utilization of adrenocortical hormones** could refer to one or a number of processes occurring in the peripheral tissues, such as enzymatic degradation of the hormone to functionally inactive metabolites, binding of the hormone by intracellular particles, transport of the hormone across cell membranes, etc. It is thus essential to study the metabolic fate of adrenocortical hormones in the peripheral tissues before one can really say whether or not increased utilization of cortical hormones occurs during stress."



## CHAPTER IV

### HYPOPHYSIO-PORTAL CIRCULATION

Lieutaud in 1742 described very small longitudinal vessels running along the stalk and these had communications with the vessels in the pituitary. In 1751 De Bordeu admitted the existence of axial vessels along the stalk and he stated that Riolan long before noted the same vessels. Zuckerman (1954) (from whose paper the above references have been collected) stated, "as we now know Lieutaud and De Bordeu were both right and wrong. De Bordeu was wrong and Lieutaud right in suggesting that the axial stalk vessels communicate with those of the pituitary below ; while De Bordeu was well justified in attacking Lieutaud's conception of the structure and function of the brain, and in doubting that a vital fluid flows from the base of the brain to the pituitary." Wenzel (1812) observed vessels connecting the infundibulum with the pituitary. Luschka (1860) wrote "here and there the vessels bulge out in the form of loops which are arranged in various ways they make their way into the interior of the infundibulum and sometimes their number is so great, that they alone constitute a loose, red substance in the interior. By these vascular loops and the productions which result thence, not only the cavity of the infundibulum is obstructed, but also the proper substance of this organ is crowded out and broken up." These are the primary capillary plexus of the pituitary portal vessels.

Popa and Fielding (1930) described that blood was collected from both lobes of the pituitary and was redistributed by a secondary capillary net in the hypothalamus. Wislocki and King (1936) and Wislocki (1937 and 1938) confirmed the presence of these vessels but contradicted the direction of flow of blood described by Popa and Fielding. According to Wislocki and King the flow of blood was from the median eminence to the pars distalis of the pituitary. Houssay, Biassoti and Sammartino (1935) first described the hypophysiportal circulation in a living toad. The circulation was from the hypothalamus to the ventral surface of the pars distalis of the pituitary. If these vessels were cut or burned, an extensive area of the anterior part of the pituitary was necrosed ; but the pars intermedia, neurohypophysis and some portion of the anterior lobe escaped as they received extra supply of blood from the brain and the basilar artery. Lascano—Gonzalez (1935) observed that after lesions of the hypothalamus anterior to the hypophysis in toads there was infarction of the pars distalis but the neural lobe escaped. Green (1947) and Green and Harris (1949) confirmed (in amphibians and living rats) the flow of blood from the median eminence to the pars distalis. Morato (1939) confirmed the direction of flow in the portal vessels from the median eminence towards the pars distalis using dyed fat embolism method. Barnett and Greep (1951), Harris (unpublished data), de Groot (unpublished data) confirmed that the flow of blood was towards the pars distalis. Green and Harris



(1947) examined this region in different mammals and came to the same conclusion as that of Wislocki. Green (1947, 1948, 1951a, 1951b) examined many types of vertebrates and found portal vessels to exist in all of them. In 1951, he said, "This study deals with 76 species, from amphioxus to man studied in serial section by a variety of methods. A hypophysioportal circulation seems constant in vertebrates from the anura to the primates and similar vessels occur in cyclostomes, fishes and salamanders."

Apart from the portal vessels, the pituitary is also supplied by systemic arteries from internal carotid artery and the venous drainage is to the adjacent venous sinuses. Harris (1948, 1950) said that most workers now agree about the absence or scantiness of the nerve supply to the pars distalis. Green (1951b) could not find any innervation of the pars distalis, "although fibers and endings were seen in the pars intermedia and fibers accompanying vessels in the pars tuberalis were common. Branches or collaterals from the tractus hypophysius ending around vessels of the primary capillary net were found constantly."

#### **Blood supply of the pituitary in different species :—**

**Fishes :—**Florentin (1936) described vessels in teleosts.

Miller (1944)—*Corydora paliatus*. Carere-Comes (1936), Ranzi (1936)—Increased vessels in the hypophyses of viviparous elasmobranchs during gestation.

Green (1951b) described the vessels of the hypophysis in elasmobranchii; branches of vessels passed in the cleft between the neuro and the adenohypophysis. From this plexus the blood supply to the neurohypophysis and the adenohypophysis was derived. This plexus also anastomosed with the vessels of saccus vasculosus.

#### **Amphibians :—**

Schobl (1882)—Salamanders.

Francis (1934)—Salamandra.

Roofe (1935, 1938)—Ambystoma.

Houssay, Biassoti and Sammartino (1935)—Toad.

Lascano—Gonzalez (1935)—Toad.

Houssay (1949)—Toad.

Craigie (1938, 1939)—Frog.

Taylor and Craigie (1938)—Frog.

Green (1947)—a variety of living amphibians.

#### **Reptiles :—**

Baumgartner (1916).

Cieslak (1945)—Garter Snake.

Green (1951b) said, "The reptilian hypophysis shows considerable variation among the different subclasses and orders but, in general, it resembles the avian gland more closely than it does the amphibian or mammalian." Green (1951b) described the blood vessels of the pituitary in Chelonia. The welldeveloped median eminence contained a portion of the primary capillary net of the portal vessels. The remaining portion of the plexus was on the surface of it. The portal vessels passed downward from the primary plexus in thick glandular tissue at the junction of the pars tuberalis and pars distalis. In the pars distalis the portal vessels broke up into secondary—sinusoidal—capillary net.



**Birds :—**In 1871 Muller described vessels entering into the pars distalis from the dura mater through a special region which he mentioned as "Oberlappen." Von Economo (1899) also mentioned the "Oberlappen." Krause (1921), Rahn (1939), and Drager (1945) observed these vessels. Green (1951b) found that the carotids anastomosed behind the pars distalis. This type of H shaped anastomosis was common in reptiles. The primary capillary net was found on the surface of the median eminence and was fed by the superior hypophysial arteries. The portal vessels entered into the pars distalis piercing a thick region of the dura. Green (1951b) said that, "this region is undoubtedly the Oberlappen." The neural lobe had independent blood supply.

#### **Mammals :—**

Morin and Botner (1941), Harris (1950), Landsmeer (1951)—**Rat.**

Morin and Botner (1941)—**Mouse.**

Morin and Botner (1941), Bruzzone (1948)—**Guinea-pig.**

Harris (1947)—**Rabbit.**

Dandy and Goetsch (1911), Wislocki (1937), Morato (1939), Bruzzone (1948), Nowakowski (1951)—**Cat.**

Dandy and Goetsch (1911), Basir (1932), Morato (1939), Bruzzone (1948)—**Dog.**

Wislocki and King (1936), Wislocki (1938)—**Monkey.**

Fuchs (1924), Nikolskaia (1929), Popa and Fielding (1930), Wislocki and King (1936), Fumagalli (1941), Morin and Botner (1941), Bruzzone (1948), Green (1948), Xuereb *et al.* (1954), McConnell (1953)—**Vascular supply of Human hypophysis.**

In the porpoise and whale the anterior and the posterior lobes of the pituitary gland are separate from each other by a thick connective tissue septum. Here also the portal vessels come from the median eminence to the anterior lobe and its position is anterior to the septum (Harris, 1947, 1950).

#### **Evolution of the hypophysio-portal circulation :—**

Green's (1951b) concept is mentioned below. This is based on the study of 76 species.

In cyclostomes and fishes he found a plexus of vessels in between neuro and adenohypophysis. In fishes the plexus was greatly folded but not in cyclostomes. In cryptobranchus the arrangement was similar but a neural lobe with independent blood supply was encountered. In other salamanders it entered into the median eminence before coming to the pars distalis and in frogs and toads, the vessels after coming out of the median eminence formed portal vessels which supplied the pars distalis through a secondary capillary net. In the reptiles, birds and mammals, hypophysioportal vessels were always found. "The only notable variations in the higher vertebrates are in the primary capillary net which may consist chiefly of a plexus between the pars tuberalis and median eminence (birds) or a system of looped vessels (as in the dog, cat or pig) or a system of skein-like vessels as in man and to a lesser extent, the rhesus monkey."



That the connexion of the pituitary with the hypothalamus is essential for the proper development of the former was understood long time back. Thus Blount (1945) and Hegre (1946) found that grafts of salamander hypophysis did not grow when the diencephalic tissue was not present. If diencephalic tissue with pituitary was transplanted, there was a better growth. Similar suggestion on the importance of this vascular connexion came also from Harris (1944), and Green and Harris (1947) for the proper functioning of the anterior pituitary. Green (1951b) mentioned, "The hypophysiportal circulation could conceivably control the activity of the pituitary in one of two ways, by regulating the blood supply to the gland or by means of one or more hormonal substances produced in the median eminence as the result of nervous activity and carried to the pars distalis there to exert an excitatory or inhibitory effect."

Green (1952) summarised the relationship between nervous system and adenohypophysis :—

- (1) Pituitary substances passed to the hypothalamus through portal vessels. This has been proved to be false.
- (2) Blood in the pars tuberalis mixed with blood in the median eminence.
- (3) Direct innervation of pars distalis.
- (4) Hypophysial substances passed between nerve fibres to hypothalamus.
- (5) Hypothalamic substances passed between nerve fibres to hypophysis.
- (6) Nerve fibres stimulated pars intermedia which then acted on the pars distalis.
- (7) Vasomotor mechanism leading to activity of the pars distalis.
- (8) Substances liberated in the median eminence found an entry into the pituitary portal vessels and thus reaching the pars distalis, activated it.

#### Regeneration of pituitary portal vessels after stalk section :—

When the pituitary stalk is severed, the regeneration of the stalk vessels is very quick until some obstacle is placed in between the stumps. Investigations have been done previously without giving any importance to this vascular regeneration and thus pituitary function was thought to be normal after stalk section.

de Groot (1952) found that in the *mouse* after stalk transection the lymphopenic response temporarily ceased but the function returned within 2 weeks. This was due to regeneration of the portal vessels. Harris (1949) and Harris and Jacobsohn (1949) found regeneration of portal vessels in *rats* within 24 to 48 hours after section.

Jacobsohn (from Harris and Fortier, 1954) found such regeneration in *rabbits*.



Donovan and Harris (1954) and Thomson and Zuckerman (1953) found this regeneration in *ferrets*.

Harris and Johnson (1950) established this to be true in *monkeys* also.

Harris (1950) studied the rate of regeneration of the hypophyseal portal vessels in rats after sectioning the stalks. The animals were killed in groups of three 1, 2, 3, 5, 7 and 10 days after operation. In all these animals regeneration of vessels occurred. "In two animals in which the stalk had been torn by a hook and in which the (anterior) hypothalamic end of the stalk had been intentionally left drawn forwards beneath the median eminence, the intervening gap had been bridged only by a leash of fine parallel running capillaries. These two animals were killed on the seventh and tenth days after operation, and the pars distalis of both, showed some central atrophy."

**Stalk section experiments in relation to ACTH discharge from the anterior pituitary :—**

Uotila (1939) found in the rat that after stalk section, adrenal enlargement following cold exposure was not prevented.

Keller and Breckenridge (1947) did not find any suppression of the adrenal hypertrophy in dogs subjected to cold after stalk section.

Cheng *et al.* (1949) and Fortier and Selye (1949) could not find any suppression of adrenal ascorbic acid depletion after histamine or surgical trauma.

Colfer *et al.* (1950) found that emotional stress produced a lymphopenia in the normal but not in the hypophysectomized rabbit. de Groot and Harris (1950) could abolish the lymphopenic response following an emotional stress stimulus in rabbits with lesions in the zona tuberalis. This was also abolished or diminished by transverse lesions in the posterior tuber cinereum or the mammillary body. Lesions in the pars distalis or pars intermedia could not abolish the response.

Harris (1952) said, "At the present time it may be taken as established that the hypophyseal portal vessels are intimately concerned with the maintenance and control of anterior pituitary activity. The exact mechanism by which they perform this function is unknown. It seems more likely that they transmit a specific humoral substance liberated into the primary plexus by nerve fibres of the hypothalamus, than that a control exists dependent on total pituitary blood supply." "It would seem likely that the feed back of hormones secreted from the target endocrines (gonads, thyroid, adrenal cortex) to the central nervous system is responsible for the behavioural patterns of various endocrine states."

Tang and Patton (1951) studied adeno-hypophyseal function in guinea-pigs after hypophyseal stalk section. Secretion of ACTH in response to cold stress was tested by measuring adrenal cholesterol content. "The results indicated that secretion of ACTH, growth hormone and gonadotrophins do not depend on an intact hypophyseal stalk."

Barnett and Greep (1951) did not find regeneration of pituitary portal vessels after pituitary stalk section in rats. There was histological evidence



of hypofunction of the pituitary gland, and the adrenals and thyroids were atrophic proving that the adrenotropic and thyrotropic hormones were subnormally released. After exposure to cold, the output of both these pituitary hormones were increased. This indicated that during stress the pituitary responded even if the direct neural or neurovascular connexion between the central nervous system and the anterior pituitary was severed. "The findings suggest that the titer of cortical and thyroid hormones in the blood appears to play the major role in the regulation of pituitary adrenotropic and thyrotropic activity."

Hume (1950) placed polyethylene film in between pituitary and hypothalamus after stalk section in dogs in order to prevent vascular regeneration. The normal eosinopenic response to stress was not blocked. His conclusion was that "the hypothalamic control of pituitary release of ACTH must be mediated by a humoral mechanism employing the general circulation for transport."

Hume (1952) found that section of the hypophysial stalk did not appear to prevent the release of ACTH secretion following stress. de Groot (1952) observed that stalk transection in mice resulted in temporary inhibition of lymphopenic response but the response returned within two weeks. This was closely correlated with the degree of regeneration of the pituitary portal vessels. A waxed paper plate between the cut ends of the stalk abolished the lymphopenic response. In these animals no portal vessels were found. There was slight adrenocortical atrophy after stalk section and well marked atrophy if portal vascular regeneration was prevented by placing waxed paper plate in between the cut ends of the stalk.

Recant *et al.* (1950) got responses from the anterior pituitary when it was devoid of its nervous and vascular connexions with the hypothalamus.

McCann (1953) observed in rats that stalk section gave rise to permanent interruption of the portal system. In these animals the responses were abolished whether the median eminence was injured or not. There was marked adrenal and testicular atrophy. There was a severe degree of anterior lobe atrophy with a butterfly shaped area of fibrosis similar to that reported by Barnett and Greep. Epinephrine eosinopenia could not be evoked in the stalk sectioned animals. The mean increase in eosinophils of six stalk sectioned animals was  $2 \pm 9\%$ . "A neurohumoral substance is released in the median eminence which normally traverses the hypophysial portal vessels and causes release of ACTH. In cases of severe stress sufficient amounts of this substance may be released to activate the anterior lobe via the general circulation." The transport through general circulation during stress was described by Hume in 1950.

Keller *et al.* (1954) concluded, "Neither adrenal cortical function nor structure is dependent upon the combined structural integrity of (a) the ventral half or more of the hypothalamus plus the hypophysial stalk and (b) the vascular channels connecting the adeno-hypophysis and hypothalamus. This is evidenced in the chronic ventral hypothalamectomized dog by the absence of (a) any tendency to adrenal insufficiency, (b) the



presence of predictable maximal eosinopenic responses associated with major surgical procedures and (c) the absence of adrenal cortical atrophy."

Montastruc (1954) studied the adrenal histology after hypophyseal stalk section in the dog.

Hume (1954) postulated two hypothalamic humoral substances; one reached the pituitary through the portal vessels and gave rise to ACTH release in the resting animal. The second substance was released during stress and reached the pituitary through the portal vessels; but in the absence of portal vessels, the second substance could utilize the systemic circulation.

Porter (1954) observed that eosinopenia produced by hypothalamic stimulation was completely prevented by pituitary stalk section in monkeys after placing tantalum foil over the sella turcica.

Zuckerman (1955) did not find any importance of portal vessels for the positive responses of the pars distalis.

Zuckerman (personal communication, 1956) said that severance of the pituitary portal vessels would almost always, but not invariably, lead to damage to the anterior pituitary, and that this might be responsible for its subsequent hypofunction. He said that "I, myself, always insist that we focus our attention, not on the evidences of damage or on hypofunction, but on those cases where, having severed the pituitary stalk and prevented regeneration of the vessels, function remains normal."

Shirley and Nalbandov (1956) did not find any significant alteration in the adrenotrophic hormone secretion after stalk transection in the laying hens.

Rothballer and Skoryna (1956) found ischaemic necrosis always in the anterior lobe after its separation from the median eminence. If a small amount of adenohypophysis was still attached to the brain, the changes were very inconspicuous or absent. The dogs kept a good health for many months in spite of some anterior lobe deficiency.

Russell (1956) studied the effect of division or obstruction of the pituitary stalk vessels in man. All three patients showed acute anterior lobe deficiency before death.

Harris and Fortier (1954) made stalk section experiments in rabbits with steps adopted to prevent vascular regeneration. There was marked atrophy and decreased activity of the adrenal cortex. There was "marked reduction, or abolition, of the effect on the thyroid and adrenal cortex of stress stimuli that act predominantly through nervous or emotional excitation.... Only slight diminution of the thyroid and adrenal cortical response to the stress of surgical trauma, or of the adrenal response to injection of large doses of adrenaline." They finally conclude, "serious consideration should be given to the suggestion that, under conditions of particularly severe stress, a chemotransmitter might be released from the median eminence in sufficient concentration to affect the pituitary via the systemic circulation."

Harris (1955) found that even after stalk section and steps taken to prevent vascular regeneration, systemic stress could still cause ACTH



discharge. This cannot coincide with the views of Hume (1952), McCann (1953), and Porter (1953) who did not find adrenocorticotrophic response to surgical trauma or injection of adrenaline after hypothalamic lesions. Harris (1955) said "at the moment there is no obvious explanation of this discrepancy."

Harris (personal communication, 1956) said that pituitary stalk section seemed to abolish the nervous reflex activation of ACTH secretion and the nervous reflex inhibition of TSH secretion. The isolated pituitary gland after pituitary stalk section still seemed capable of changing its slight secretion of ACTH and TSH in response to physical damage. "I can only guess that this is due to some change in the composition of the general circulating systemic blood (may be histamine liberation from traumatized tissues which can act directly on the pituitary cells)."

Fortier, Harris and McDonald (1957) stressed the importance of hypophyseal portal vessels as functional link between the hypothalamus and the anterior pituitary. Permanent interruption of these vessels lowered the basal rate of secretion of ACTH, prevented the nervous reflex activation of ACTH in response to stress, and abolished the gonadotrophic hormone secretion. Though the secretion of gonadotrophic hormone was entirely dependent on intact hypothalamohypophyseal connexions, yet residual secretion of ACTH and modified secretion of it by certain types of stress remained even in the absence of these connexions.

I conclude this review by quoting Selye's (1956) remark "Certainly the vast majority of evidence available today does seem to show quite clearly that through nerve fibers and blood vessels the neurosecretions of the hypothalamus travel down to the pituitary and influence the secretion of the latter."

#### **Pituitary Grafting Experiments and Secretion of ACTH from the Grafts :—**

Cheng *et al.* (1949) did the auto and homotransplantation experiments with anterior pituitary tissue placed into the eye, spleen or sella turcica of adult hypophysectomized rats. ACTH was discharged from the transplanted pituitary in response to histamine injection though the amount discharged seemed to be less than normal. There was decrease in the weight of the adrenals and "in most instances as great as that which occurred in the hypophysectomized animals without pituitary grafts."

McDermott *et al.* (1950) experimented with hypophysectomized male rats by placing pituitary grafts into the anterior chamber of the eye. Though the adrenal glands were partially atrophied, subcutaneous injection of hypertonic saline or epinephrine injection either subcutaneously or into the graft containing eye, led to eosinopenia.

Schweizer and Long (1950) found partial maintenance of the adrenal cortex by anterior pituitary grafts.

Sayers (1950) also did not find any importance of neural or neurovascular link between the hypothalamus and the anterior pituitary for the ACTH discharge.



Harris and Jacobsohn (1951) examined 151 hypophysectomized adult rats after placing anterior pituitary grafts in different situations *i.e.* under the temporal lobe and under the median eminence. The animals with grafts placed under the temporal lobe had ovaries, reproductive tract, adrenals and thyroid atrophied. In the animals with grafts placed under the median eminence the above structures appeared normal. In the above two groups, the vascularity of the grafts was all right and there was no discrepancy in the size of the grafts. The vascular supply to the temporal lobe grafts was from cerebral vessels and that of the grafts placed under the median eminence was from the hypophysial portal vessels. Acidophil, basophil and chromophobe cells were found in the well differentiated median eminence grafts but the temporal lobe grafts contained only uniform small cells. He said, "It appears, then that ACTH secretion by grafted anterior pituitary tissue is only retained at a normal level if the tissue is vascularized by the hypophysial portal vessels, and that if the tissue is in a site remote from the hypothalamus, partial or complete adrenal atrophy occurs. This indicates that variation in the blood levels of epinephrine or of adrenal cortical hormone does not stimulate ACTH secretion by direct action on anterior pituitary cells." Harris and Jacobsohn (1952) found similar results as Harris stated in 1951. Fortier and Selye (1949) noted release of ACTH from ocular pituitary grafts in hypophysectomized rats in response to cold and unilateral adrenalectomy.

When foetal pituitary glands were implanted into the anterior chamber of the eye of hypophysectomized male rat, viable, proliferating and highly differentiated pituitary lobes were found. The anterior lobe was mainly composed of chromophobic elements but contained sparse granulated alpha cells (acidophils), well granulated beta cells and rare pale staining delta cells (Goldberg and Knobil, 1957). They also found evidences of slight secretion of ACTH by the implants following acute stress.

Fortier (1951) studied the response of the grafted pituitaries (in the anterior chamber of the eyes) in hypophysectomized rats to stimuli. The stimuli were systemic (adrenaline, cold, histamine) and neurotropic (sound, immobilization). The ocular grafts were richly vascularized. The histology showed predominant chromophobic elements with rare acidophils and fewer basophils. The adrenal glands were atrophied in a much smaller degree and adequately maintained. He found response (eosinopenia) from the grafted pituitaries by adrenaline, cold, histamine. Sound and immobilization did not give rise to any response in the grafted animals. He concluded, "These results suggest a dual regulation of ACTH release, one purely humoral, in response to systemic stimuli, the other probably neurohumoral mediated by the hypothalamo-hypophysial neurovascular pathways, and coming into play under the influence of nervous and emotional stimuli."

Fortier (1952) confirmed the dual control of the ACTH release (Fortier, 1951) and he found that subconjunctival injections of adrenaline and histamine into the graft-containing eye gave rise to pronounced eosinopenia. Local cold, dibenamine (when applied to graft-containing eye)



and adrenaline and histamine (injected into the intact eye) did not give rise to eosinopenia.

From the survey of the grafting experiments it is found that the conclusion is not unanimous regarding the importance of the hypothalamo-hypophysial vessels in the activation of the anterior pituitary to discharge ACTH. Harris and Jacobsohn (1951) found atrophy of the adrenals in animals in which the grafts were placed in areas other than the median eminence. Others found atrophy of the adrenals in grafted (ocular) animals but the grafted pituitaries secreted ACTH when stimulated and thus pituitary portal vessels were not required. Fortier (1951) found the adrenal glands to be atrophied in a much smaller degree and adequately maintained. He has, however, tried to cover the discrepancy by explaining the dual regulation of ACTH release—one, purely neurotropic stimulus required the portal vessels for its activation on the anterior pituitary, the other, systemic stimulus was "mediated through various humoral changes induced by stress and acting either independently or concurrently on the pituitary to elicit the release of ACTH". (Fortier, 1952).

The present investigation has been taken up with a view to study whether corticoid output increases in response to immobilization and injuries in dogs with the stalk sectioned and measures taken to prevent vascular regeneration.

#### Materials and methods :—

(1) Male dogs of 10 to 15 kg. in weight have been used in this experiment. The daily diet was kept constant and water was given freely.

(2) Pituitary stalk section was done by the transtemporal route and precautions taken to prevent regeneration of the portal vessels (Rothballer and Skoryna, 1956). The dogs were anaesthetized with pentobarbital.

(3) Histological examination of the operated area has been done after India ink injection at the termination of the experiment and some preparations have been stained by haematoxylin and eosin.

(4) Cannulation of the right adrenal vein for the collection of the adrenal venous blood was done after the method of Hume and Nelson (1955). Adrenal venous blood samples were collected in heparinized tubes. After immediate centrifuging, the plasma was preserved in cold for the determination of 17-hydroxycorticosteroid after the method of Silber and Porter (1954).

(5) Stresses include :—

- (a) Purely psychic stress as immobilization for 2 hours.
- (b) Fracture of right femur.

#### Results :—

During the cannulation of the adrenal vein in normal dogs, the 17-hydroxycorticosteroid output is high when compared to the post-cannulation values. In the five dogs examined, the 17-hydroxycorticosteroid



output ranges from 12.6 to 18.2 gamma per minute with an average of 15.4 gamma per minute for all (5) dogs in the cannulation period. In the second postcannulation day, the values range from 1.6 gamma per minute to 2.3 gamma per minute with an average of 2 gamma for all dogs. When these dogs are subjected to a purely psychic type of stress as immobilization, the 17-hydroxycorticosteroid output rises and the values are from 12.6 to 14.8 gamma per minute with an average of 13.6 for all 5 dogs. Thus it is found that the corticosteroid output during psychic stress approximates towards the value during cannulation but is still lower than it.

In the pituitary stalk sectioned dogs during the psychic stress as immobilization, the 17-hydroxycorticosteroid output is from 1.2 to 2.6 gamma per minute with an average of 1.7 for all 8 dogs. Thus while in normal dogs the corticosteroid output is 13.6 gamma per minute during immobilization stress, it is 1.7 in the pituitary stalk sectioned dogs (during the same type of stress) which does not differ from pre-immobilization (1.9) or post-immobilization (1.8) values, proving thereby that during purely psychic type of stress pituitary stalk is essential for the proper adrenocortical response.

In a few animals, which had well marked vascular regeneration across the pituitary stalk sectioned area, there was good adrenocortical response after immobilization stress.

In the pituitary stalk sectioned dogs during the right adrenal vein cannulation, the 17-hydroxycorticosteroid output varied from 12.8 to 20.2 gamma/minute with an average of 16.3 for all (8) dogs. This high level came down on the 1st and 2nd post-cannulation days. On the 1st post-cannulation day the average figure for all 8 dogs was 3.3 gamma per minute which on the second day came down to 1.9 gamma per minute. After fracture the 17-hydroxycorticosteroid level was high and in the eight dogs the values were from 10.6 to 19.2 gamma per minute with an average of 16.9 for all eight dogs. On the next day after fracture the values came down to an average of 2.9 gamma per minute. The average 17-hydroxycorticosteroid output after fracture was nearly of the same magnitude as that found during cannulation of the right adrenal vein.

#### Histological results :—

- (a) **Pars distalis**—The pars distalis in the stalk sectioned animals showed a small area of ischaemic necrosis in the immediate post-operative period. The remaining portion was well vascularized.
- (b) **Pars intermedia**—appeared to be hypertrophic.
- (c) **Neural lobe**—showed usual degeneration and atrophy.
- (d) **Median eminence**—In the immediate post-operative period there was thrombosis in the primary plexus of the hypophysial portal vessels in the median eminence. The median eminence was found to be enlarged in this group when compared to that in control animals.



### 17-Hydroxycorticosteroid output in normal dogs in response to immobilization stress

Dog No.	Wt. in Kg.	During cannulation	Post-cannulation period,	During immobilization,
17-Hydroxycorticosteroid output gamma/minute.				
59	14	16.1	2.3	14.8
60	14.5	18.2	1.6	12.6
61	15	12.6	2.4	13.2
62	13	17.5	1.8	14.2
63	10	12.8	1.9	13.2
Average		15.4	2	13.6

### 17-Hydroxycorticosteroid output in pituitary stalk sectioned dogs after immobilization stress and fracture

Dog No.	Wt. in Kg.	During cannulation.	1st Post-op. day.	2nd Post-op. day.	Immobilization stress		Fracture	1st day after fracture.
					During	After		
17-Hydroxycorticosteroid output gamma/minute								
64	12.5	15.5	6.8	2.3	2.6	2.1	16.6	5.4
65	15	18.6	5.2	1.8	1.5	1.7	17.7	4.6
66	14.5	16.2	2.1	1.9	2.5	1.6	16.8	3.4
67	12	14.3	1.4	2.1	1.8	1.4	18.6	1.8
68	11	12.8	10.2	1.6	1.2	2.1	19.2	1.6
69	13	13.9	6.4	2.3	1.7	2.1	10.6	1.7
70	14	19.4	2.3	1.8	1.4	1.8	17.4	2.3
71	14.5	20.2	1.2	1.6	1.5	1.7	14.4	2.4
Average		16.3	3.3	1.9	1.7	1.8	16.9	2.9

### Discussion :—

There is difference of opinion regarding the importance of hypophysial portal vessels in the ACTH secretion from the anterior pituitary. Uotila (1939), Keller and Breckenridge (1947), Cheng *et al.* (1949), Fortier and Selye (1949), Tang and Patton (1951), Barnett and Greep (1951), Hume (1950, 1952), Recant *et al.* (1950), and Keller *et al.* (1954) found adrenocortical response in stalk sectioned animals using different types of stress. Others found blocking of the adrenocortical response to stress after pituitary stalk section (de Groot and Harris, 1950 ; Harris, 1952 ; de Groot, 1952 ; Porter, 1954 ; Harris and Fortier, 1954). Most of the earlier experi-



ments have been done without giving any importance to the vascular regeneration which occurs after stalk section if some obstacle is not placed in between the cut ends of the stalk. The vascular regeneration has been shown to occur in the rat (Harris, 1950 ; Harris and Jacobsohn, 1950), mouse (de Groot, 1952), rabbit (Jacobsohn, 1954), ferret (Thomson and Zuckerman, 1954 ; Donovan and Harris, 1956), and monkey (Harris and Johnson, 1950).

Hypophysectomized rats, bearing extrasellar implants of pituitary, showed adrenocortical responses (Cheng *et al.*, 1949 ; Fortier and Selye, 1949 ; McDermott *et al.*, 1950). Fortier (1951) suggested from grafting experiments a dual regulation of ACTH release :—

- (a) One purely humoral, in response to systemic stimuli.
- (b) The other, probably neurohumoral required hypothalamohypophysial neurovascular pathways in nervous and emotional stimuli.

Fortier *et al.* (1957) came to the same conclusion regarding the dissociation in the response to different stressing procedures.

In the present investigation similar type of dissociated response has been found in dogs by measuring the adrenal venous corticoid output. Purely psychic stress as immobilization, in dogs, requires the presence of the hypothalamo-hypophysial neurovascular connexions as in the absence of this pathway, the adrenal venous 17-hydroxycorticosteroid output does not rise but in those animals in which the regeneration of vessels occurred, the corticosteroid output in response to the psychic stress was high.

In the stalk sectioned dogs, the 17-hydroxycorticosteroid output after fracture was nearly of the same magnitude as that found during adrenal vein cannulation. But in the normal dogs (average of 20 dogs) the response after fracture was greater than that during adrenal vein cannulation (during cannulation 17-hydroxycorticosteroid output—19.5 and after fracture—26.9 gamma per minute). Thus there is a difference in adrenocortical response in fracture in between normal and stalk sectioned animals. Adrenocortical response is not so high in the stalk sectioned animals. The reason may be as follows :—

- (a) Hypofunction of the anterior lobe tissue because of partial jeopardization of vascular supply ; but this is impracticable as it has been shown by Ganong and Hume (1956) that three-quarters of the anterior pituitary may be removed in dogs without any endocrine abnormality. Smith (1932) showed that in rats 10 per cent of normal anterior pituitary volume was just for maintaining some ovarian activity and full sexual functions will be maintained by 30%.
- (b) McCann (1953) suggested that a neurohumoral substance is released in the median eminence which normally passed through the hypophysial portal vessels and caused discharge of ACTH. In severe stress, this substance may also pass through the general circulation to activate the anterior lobe. Such a suggestion is a possibility in explaining the adrenocortical response found after adrenal vein cannulation and fracture in stalk sectioned animals.



- (c) From the injured sites of fracture and operation histamine is liberated which may act directly on the anterior pituitary to stimulate ACTH secretion. That histamine is liberated after fracture has been dealt with in the relevant chapter and thus this is also another possibility.
- (d) According to Long's epinephrine hypothesis, liberated epinephrine in response to fracture and operations may act directly on the adenohypophysis to discharge ACTH.

Following stalk section in dogs, ischaemic necrosis affecting a small part of the anterior pituitary occurs. This has been shown also by Rothballer and Skoryna (1956) in dogs. Lascano-Gonzalez (1935) first observed such infarction in toads. Anterior pituitary necrosis following stalk section has been found by many in different vertebrates (Campbell and Harris, 1957; Westman, Jacobsohn and Hillarp, 1943; Daniel and Prichard, 1956; Benoit and Assenmacher, 1953; Shirley and Nalbandov, 1956; Russell, 1956; LeBeau, Perrault and Testard, 1956; and others). Though a portion of the anterior lobe undergoes necrosis, still the remaining portion is sufficient to discharge ACTH. There is atrophy of the neural lobe. Enlargement of the pars intermedia occurs after stalk section. This has been shown also by Brooks (1938), and Barnett and Greep (1951).

The median eminence and the proximal stump of the stalk is found to be enlarged in the stalk sectioned dogs. The vascular regeneration was prevented across the site of stalk section. Magoun *et al.* (1939) mentioned an apparent hypertrophy of the median eminence in the stalk sectioned monkeys. Campbell and Harris (1957) have shown such hypertrophy of the median eminence in the stalk sectioned rabbits. Median eminence hypertrophy has been mentioned by Benson and Cowie (1956) in the partially or completely hypophysectomized rats. Accumulation of neurosecretory substance proximal to the area of stalk section has been observed in different vertebrates by many (Stutinsky, 1951, 1954; Hild, 1951; Mazzi, 1954; Benoit and Assenmacher, 1954; Scharrer and Wittenstein, 1952; Roy, 1957). Experiments on invertebrates also show the same feature (Scharrer and Scharrer, 1954; Passano, 1953; and others).

The neurosecretory substance is accumulated proximal to the area of section because it cannot pass distally. Increased activity in the proximal stump and in areas even more proximal to it has been observed by many (Sato, 1928; Lloyd and Pierog, 1955; Moreno *et al.*, 1955; Barnett, 1954; and Billenstien and Leveque, 1955). The enlarged median eminence and the proximal stump in dogs in the present study may be due to an accumulation of neurosecretory substance which cannot pass to the hypophysis along its well-established routes. A similar feature has been observed by me in guinea-pigs after pituitary stalk is sectioned and vascular regeneration is obstructed.

#### Conclusion :—

- (1) Purely psychic stress as immobilization in dogs does not give rise to increased adrenocortical activity, when the pituitary stalk is sec-



tioned and regeneration of vessels prevented. However, when regeneration occurs, increased adrenocortical response is found.

(2) Adrenocortical response after adrenal vein cannulation and fracture occurs in the pituitary stalk sectioned dogs. Fracture has been used here as stressing procedure, as this has not been used previously along with direct measurement of the adrenocortical activity by the study of the adrenal venous 17-hydroxycorticosteroid output.

(3) Findings 1 and 2 confirm the previous findings regarding the dissociated response from the two different types of stress but carried on by a direct measurement of the adrenal venous corticoid output.

(a) purely psychic stress requires the presence of the hypophysial portal vessels.

(b) operation for adrenal vein cannulation or fracture gives rise to increased adrenocortical response even in the absence of the hypophysial portal vessels. Causes have been discussed before.

(4) Atrophy of the neural lobe, ischaemic necrosis of the anterior lobe, enlargement of the pars intermedia and proximal stump of the stalk and median eminence have been observed.



## CHAPTER V

### HISTAMINE CONTENT OF THE FRACTURE HAEMATOMA AND BLOOD IN FRACTURE CASES AND THE STUDY OF HISTAMINE CONTENT OF THE BLOOD OF BURN PATIENTS

Shovanec (1947) thought that the rise in histamine content of blood played an important part in the catabolic phase. In the pathogenesis of shock caused by trauma, attention was given by many to histamine and histamine like compounds (Cannon, 1922 ; Dale, 1920, 1932 ; Johnson and Blalock, 1931 ; Schneider, 1932 ; Barsoum and Smirk, 1936 ; Allen, 1939 ; Chambon *et al.*, 1943 ; Cicardo, 1947 ; Csaiky and Magyary-Kossa, 1947). Selye (1950) said, "yet some investigators believe that in the final analysis it is nevertheless histamine liberation that initiates the A—R syndrome although, under certain circumstances, increased histamine destruction may overcompensate for increased formation. We have also considered this possibility but took care to emphasize that there is no irrefutable evidence to support it and that numerous other metabolites might be invoked with almost equal justification." Increased blood histamine level following burns has been reported in man by Barsoum and Gaddum (1936), Code and MacDonald (1937), Rose and Browne (1942), and in animals by Behrmann *et al.* (1946), Kisima (1938). The early rise is followed by a fall to normal or below normal levels.

Regarding the part played by histamine in the mechanism of ACTH secretion, Semonsen and Sawyer (1954) concluded, "subcutaneous histamine acts as a non-specific stressing agent whose effect on the hypophysis is mediated, at least in part, via the adrenal medulla, whereas intravenous histamine may act directly upon the hypophysis." Schnitzer (1954, 1955) supported the theory that histamine liberation from injured tissues may start the G.A.S. Sawyer (1955) came to the conclusion that stimulation of the hypophysis by histamine was exerted via rhinencephalic pathways and not by a direct action on the adenohypophysis as a chemical mediator of hypothalamic activity. Fuche and Kahlson (1957) found that physiological quantity of histamine in the general circulation was an effective stimulus for increased secretion of ACTH from the adenohypophysis.

Watson Jones (1943) mentioned about the histamine and acetylcholine content of the fracture site and these caused vasodilatation and hyperaemia of the bone ends. The bone is a good source of histamine as was proved by Code and Jensen (1941). They found that the histamine content of the red or cellular marrow exceeded the concentration found in the blood of guinea pigs, rabbits, cats, dogs, horse and a cow.

In the present investigation, histamine contents of the fracture haematoma and blood from fracture cases and histamine content of the blood from burn patients have been studied on different days after trauma and the relation of the histamine content to the blood eosinophils has been noted with a view to assess the status of the adrenocortical activity.



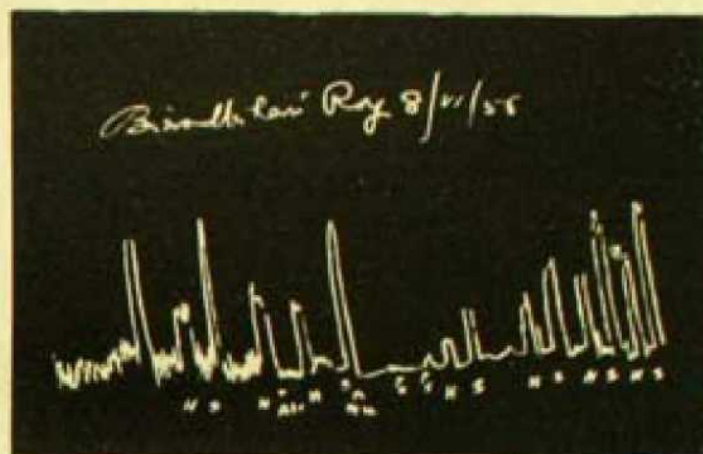


Fig. 12.—Guinea-pig's ileum. Atr. =  $1:10^7$  atropine sulphate. H =  $0.5\mu\text{g}$ . histamine. AH =  $6\mu\text{g}$ . antihistamine. S =  $0.2\text{c.c.}$  of the extract of fracture haematoma from Case M.

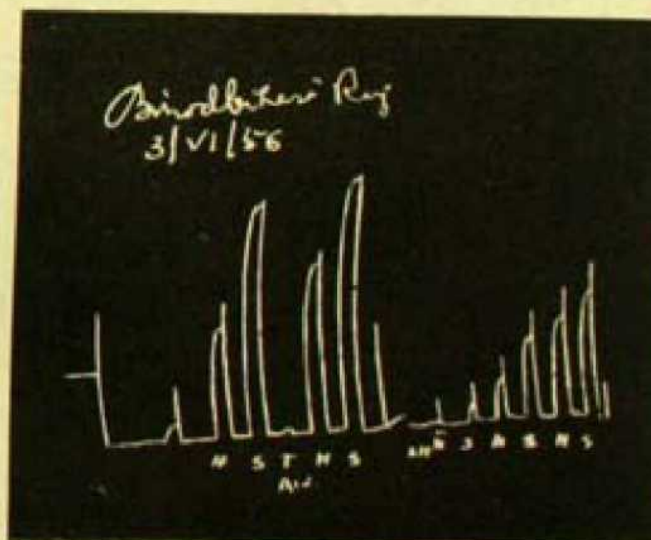


Fig. 13.—Guinea-pig's ileum. Atr. =  $1:10^7$  atropine sulphate. H =  $0.5\mu\text{g}$ . histamine. AH =  $6\mu\text{g}$ . antihistamine. S =  $0.2\text{c.c.}$  of the extract of fracture haematoma from Case B.



### Materials and methods :—

Histamine estimation has been done by biological assay method. The extraction process of fracture haematoma or blood has been carried out after Code's modification of Barsoum and Gaddum's method (1937).

Aspiration from fracture haematoma was done under local anaesthesia from femoral shaft fractures. About 10 c.c. was aspirated. From each case (total 10 cases) two aspirations on two different days were done—one on the day of fracture and another subsequently. (This did not materially affect the union of the bone.) On the same occasion of haematoma aspirations, 10 c.c. blood from the vein in the cubital space was also drawn. The blood and the content of the fracture haematoma was extracted and assayed for histamine. 10 c.c. blood was also drawn from the vein in the cubital space of each of 10 burn patients on different days after trauma. Eosinophil count of the blood of these patients was done to correlate the adrenocortical activity with the alteration in the histamine content of the fracture haematoma and blood in fracture cases and the histamine content of the blood in burn patients.

### Results :—

The histamine content of the fracture haematoma declines as the day after fracture increases. On the first day after fracture, the histamine content of the haematoma was greater than that found in the blood of the same patient at the same time of examination. The histamine content of the blood, from the high level attained after trauma, diminished and came within normal limits as the day after fracture increased. The period of eosinopenia corresponded with the period when the blood histamine was high and also the histamine content of the fracture haematoma was high. Similarly high level of histamine in the blood of burn patients corresponds well with the period of eosinopenia and so with increased adrenocortical activity.

Code and Mitchell (1957) studied the possible relationships between the concentration of histamine and the number of blood eosinophils and basophils of adrenalectomized dogs, intact guinea pigs and healthy humans. Cortisone was used in each species. In humans between 0.01 and 0.02  $\mu$ g. histamine/ml. blood would be present even when all the eosinophils were absent from the blood. According to them, histamine may be present outside the eosinophils in the blood of normal persons and also in the blood of dogs and guinea pigs.

In the present investigation it has been found that blood eosinophils are very low or zero when the histamine content is high in the blood after fracture and burn in humans. Gradually the histamine content is lowered in the blood and the number of eosinophils rises as the patients' condition improves. The increased histamine content in the absence of eosinophils in the blood is due to the increase of histamine in the extracellular portion of the blood.



## CHAPTER VI

### HYPOTHALAMUS AND ACTH SECRETION

#### Stimulation experiments :—

Remote control stimulation of the hypothalamus was done in dogs and it was found that marked eosinopenia occurred rather after stimulation of the anterior hypothalamus than the posterior one (Hume and Wittenstein, 1950).

Stimulation of the anterior hypothalamus leads to marked eosinopenia. The exact location was just to the right of the midline in the floor of the hypothalamus and it was posterior to the optic chiasma but anterior to the stalk (Hume, 1952).

Hume (1953) demonstrated that after stimulation of the median eminence and posterior tuber cinereum of the dogs there was eosinopenia indicating the release of ACTH from the pituitary. This happened in completely sympathectomized dogs meaning thereby that epinephrine release was not responsible. Stimulation of other hypothalamic areas did not give rise to eosinopenia.

Shimazu *et al.* (1954) stimulated the sympathetic centre in the hypothalamus and found neurosecretion in the hypothalamic and hypophysial blood vessels and also in the third ventricle. Stimulation of the parasympathetic centre in the hypothalamus leads to suppression of the secretion and axons contained the secretions.

Anand and Dua (1955) experimenting on unanaesthetized cats with permanently implanted multilead electrodes found that stimulation of the anteromedial regions of the median eminence leads to maximum eosinopenia and so these hypothalamic areas were concerned with ACTH secretion. Stimulation of the lateral part of the posterior tuberal area gave rise to eosinophilia due to sympathetic action leading to splenic contraction.

de Groot and Harris (1950) performed remote control stimulation of the hypothalamus and pituitary gland in the conscious, unrestrained rabbits.

**Areas from where positive response (in the form of 3rd hour maximum lymphopenia) was achieved :—**

Posterior region of the tuber cinereum, mammillary body and neurohypophysis.

**Negative response areas included :—**

1. High in the tuber cinereum, 1.5 mm. above the infundibular stem.
2. Anterior part of the tuber cinereum including the median eminence and the supraoptico-hypophysial tract.
3. Lateral region of the tuber cinereum.
4. The infundibular stem.
5. The pars and zona tuberalis.
6. The pars intermedia.
7. The pars distalis of the pituitary gland.



There was no alteration in the lymphopenic response in rabbits with lesions in the posterior part of tuber cinereum after bilateral cervical sympathectomy was done in these animals.

In the thesis of de Groot (1952) there are two diagrams on pages 44 and 45 showing electrical excitability and the effect of lesions in the hypothysial region. He has mentioned there that electrical stimulation of various parts of tuber cinereum might lead to increased anterior pituitary secretion. Similarly lesions in various parts of tuber cinereum were effective in diminishing or abolishing anterior pituitary secretion.

Stimulation of the tuberal and mammillary areas in cats resulted in eosinopenia. Surrounding regions had no modifying influence (Porter, 1953). This finding of Porter (1953) concurred with that of Porter (1952) in that increased electrical activity could be induced in posterior hypothalamus after application of stressing agents. Porter (1954) confirmed the above findings in monkeys and moreover he found certain inhibitory or augmentatory areas in the cerebrum which could modify the stress response.

**Lesion experiments :—**(Relation of hypothalamus to ACTH secretion only has been presented).

Hume (1949) found that anterior hypothalamic lesions in the supra-optic nucleus gave rise to diabetes insipidus, but it did not interfere with normal eosinopenic response to stress. Localized electrolytic lesions in another area of the anterior hypothalamus abolished the usual eosinopenic response to stressing agents though the pituitary was intact. He said that a humoral mechanism was situated in the anterior hypothalamus which could release a substance, when stimulated, and this would then act on the anterior pituitary to liberate ACTH.

When paramedian lesions were made in the anterior hypothalamus and at the junction of the middle and posterior hypothalamus, the response to epinephrine and insulin stopped and the response to operative trauma decreased. Lesions in the supraoptic nuclei or lateral or unilateral median hypothalamic lesions did not alter the response to stress (Hume and Wittenstein, 1950).

Hume (1952) found that lesions involving the anterior portion of the median eminence of the tuber cinereum or the posterior portion of the tuber and the anterior portion of the mammillary bodies blocked the eosinopenic response after epinephrine, insulin or mecholyl. Response to operative trauma was blocked also in lesions located in the anterior portion of the median eminence. He further said that there is as yet no explanation of the observation that the animals begin to respond again after a long period of time.

Hume (1953) made lesions in 89 dogs. 28 dogs were tested for responses to operative trauma.

12 animals showed normal responses.

16 animals showed abnormal responses.

Of the 16 animals showing abnormal responses 6 were not killed.

Of the 10 animals sacrificed—

1 had infarct of the anterior pituitary.



7 had lesions involving the median eminence.

2 had lesions involving the mammillary body and the posterior part of the tuber cinereum.

Of the animals still living 5 were thought to have median eminence lesions and one had lesions of the mammillary body and posterior tuber cinereum. Of the 12 animals showing normal response to operative trauma after hypothalamic lesions 9 were examined. Some had incomplete lesions of the median eminence or mammillary body. 2 animals did not show normal response and these had lesions of the posterior part of the tuber cinereum, infundibulum and the anterior portion of the mammillary bodies. One animal showing normal response had less extensive lesion of the mammillary body and posterior tuber cinereum. He said, "it seems clear that the median eminence must be intact for the normal release of ACTH following trauma."

Ganong and Hume (1954) studied absence of stress induced and compensatory adrenal hypertrophy in dogs with hypothalamic lesions. They found that normal animals and animals with hypothalamic lesions sparing the median eminence showed a 20% increase in the right adrenal size two weeks after left adrenalectomy. There was no compensatory hypertrophy in the dogs having destruction of the median eminence. Dogs having one half or more destruction of the median eminence did not get adrenal atrophy even after a long time and this occurred in hypophysectomized dogs.

Ganong and Hume (1955) concluded that an intact median eminence was required for the increase in ACTH secretion after stress, but it was not required for the decrease which followed steroid injection.

Nelson (1956) mentioned that normal dog with operative trauma secreted large amounts of 17-hydroxycorticosteroids and had measurable amount of ACTH in the peripheral blood, but the dog with destruction of the anterior median eminence showed low levels of corticoid secretion after trauma and had not measurable ACTH in the blood. "There appeared to be a basal secretion of ACTH in the dog with the hypothalamic lesion, however, so although response to stress was prevented, the adrenal cortex continued to secrete at a low level."

Ganong *et al.* (1955) studied the effect of hypothalamic lesions on plasma 17-hydroxycorticoid response to immobilization in the dog. It was concluded that hypothalamic lesions involving the median eminence could block the rise in plasma corticoids seen in response to immobilization stress in the dog.

Slusher and Roberts (1956) studied the effect of hypothalamic lesions on adrenal ascorbic acid change in response to stress in the male rat. They concluded that lesions of the tuber cinereum and anterior border of the mammillary bodies gave rise to complete block in adrenal ascorbic acid response to operative stress of laparotomy. "Furthermore these blocks occurred in rats with high water intake although the hypothalamic hypophyseal tract and median eminence remained intact."

Laqueur (1954) studied the adrenocortical hormone output in the adrenal venous blood and examined the endocrine organs in cats after



various hypothalamic lesions. In cats having median eminence lesions there was accumulation of lipids in the outer fascicular zone and the adrenocortical hormone in the blood was one-third the amount found in normal animals. The ovaries in these lesioned animals were small. Animals with lesions in the mammillary bodies had adrenals with marked lipid depletion from the outer fasciculata and adrenocortical hormone content in the adrenal venous blood was within normal reach. The ovaries in these animals were normal. He concluded, "that lesions in the mammillary body region do not interrupt the hypothalamic influence upon ACTH release from the anterior hypophysis."

Laqueur *et al.* (1955) said, "it would seem therefore that it is the acute release of ACTH in response to a stressful stimulus which is subject to hypothalamic control."

Tislow (1954) found that intact hypothalamus was required for the adrenal responsiveness to corticotrophin due to a functional relationship between the hypothalamus and the corticotrophic target organ.

McCann and Brobeck (1954) said that supraoptico-hypophysial tract may play a role in the regulation of ACTH secretion by release of anti-diuretic hormone into the hypophysial portal vessels.

Fulford and McCann (1955) found that compensatory adrenal hypertrophy occurring after unilateral adrenalectomy was suppressed with the production of diabetes insipidus by hypothalamic lesions in rats.

McCann and Sydnor (1954) studied blood and pituitary adrenocorticotrophin in adrenalectomized rats with hypothalamic lesions. The blood ACTH level of adrenalectomized, DCA treated (for 2 weeks) rats subjected to ether anaesthesia and bleeding was 11 mu. per 100 ml. Hypothalamic lesions which were suitably placed, blocked this rise in blood ACTH. Pituitary ACTH was approximately 50% of that found in adrenalectomized rats without lesions. The effective lesions blocked the supraopticohypophysial tract as this was evident from the marked diabetes insipidus.

Soulairac *et al.* (1953) studied the adrenal histology after hypothalamic lesion.

Montastruc (1954) found that four completely adrenalectomized dogs without any corticoid or sodium chloride were maintained well by severing the supraopticohypophysial tract. It was said that adrenal insufficiency did not matter much because the antidiuretic hormone secretion was suppressed. In these animals, adrenalectomy could greatly ameliorate the diabetes insipidus occurring as a result of cutting the supra-optico hypophysial tract.

Kuesco and Seitelberger (1955) reported a case of granuloma infiltrans which occurred in a lady, 25 years old. The granuloma destroyed the infundibulum and the adjacent hypothalamic centres. Though the anterior pituitary was histologically perfectly normal, yet there was diabetes insipidus, adreno cortical atrophy, and no apparent change in the adrenal medulla was noted. There was thymicolymphatic hyperplasia, and sclerosis of the thyroid, pancreas, ovary and serous hepatitis were found.



Moeri (1951) found adrenal hypoplasia in anencephaly where the hypothalamohypophysial neurosecretory system was not present but the structure of the anterior pituitary was perfect. This means that in the absence of the hypothalamus, the anterior pituitary could not pour ACTH sufficient to maintain the adrenal cortex.

Anand *et al.* (1954) said that in rats "the hypothalamic centres which control ACTH secretion from anterior pituitary (nervous phase) in response to stress are located bilaterally in the medial part of the anterior hypothalamus in the plane of the paraventricular nuclei and the area just posterior to it i.e., the anterior and medial part of the median eminence in the plane of the ventromedial nuclei. Lesions in these regions abolish the eosinopenic response. Paraventricular nuclei do not take part in this. Lesions in other hypothalamic regions do not change the response."

Lynch *et al.* (1952) found that after ventral hypothalamectomy (complete removal of the proximal hypophysial stalk and of the adjacent ventral half of the hypothalamus) in the dog there was no alteration in the eosinopenic response to surgery.

Keller *et al.* (1954) established that "neither adrenal cortical function nor structure is dependent upon the combined structural integrity of—

- (a) the ventral half or more of the hypothalamus plus the hypophysial stalk, and
- (b) the vascular channels connecting the adenohypophysis and hypothalamus. This is evidenced in the chronic ventral hypothalamectomized dog by the absence of
  - (a) any tendency to adrenal insufficiency,
  - (b) the presence of predictable maximal eosinopenic responses associated with major surgical procedures, and
  - (c) the absence of adrenal cortical atrophy."

Egdahl *et al.* (1958) mentioned that after stepwise removal of the brain down to the hypothalamus in dogs there was no diminution in the maximal adrenocortical response after operative trauma. They used the following types of preparations :—

- (a) decorticated dogs,
- (b) decorticated dogs with removal of basal ganglia, hippocampus and fornix
- (c) dogs with thalamus removed and hypothalamus intact, and
- (d) total removal of diencephalon and higher areas.

After the hypothalamus was removed, the increased adrenal corticoid secretion came down to basal level even after mesenteric traction.

McCann (1953) made lesions in the median eminence of the rat. This procedure completely blocked the adrenal ascorbic acid depletion which normally occurred in the other adrenal within one hour after unilateral adrenalectomy. Eosinopenia after epinephrine injection was also blocked. There was no change in the anterior pituitary and the adrenal cortex.



Partial lesions involving the median eminence and the adjacent areas and lesions of the mammillary bodies or paraventricular nuclei did not abolish the normal response.

Endrőczy and Mess (1955) made lesions in the rostral group of nuclei of the hypothalamus in rats with the help of stereotaxic apparatus. This did not alter the working of the pituitary-adrenal system. After lesions in the tuber cinereum and mammillary group of nuclei, stress did not lead to lymphopenic reaction, nor there was adrenal ascorbic acid depletion. After the lesion of the tuberomammillary group of nuclei, compensatory hypertrophy of the adrenal as a result of unilateral adrenalectomy did not take place. It is quite possible that the tuberal group of nuclei of the hypothalamus controls the ACTH secretion of the anterior lobe by the neurohumoral path.

Barnett and Mayer (1954) made bilateral, minute electrolytic lesions in the ventromedian nuclei of the hypothalamus of 38 adult female rats. Twelve of the rats randomly selected, had adrenals and thyroids of normal weight and histology. The anterior pituitary glands had normal cell populations but in some, degranulation of the acidophils was encountered. The thyroid and adrenal responses of 12 hypothalamic rats after exposure to cold (4°C) for 7 days were normal.

McDermott *et al.* (1950) used adrenal demedullated rats, chronic spinal rats and rats with lesions in the thalamus and hypothalamus and they concluded that in these animals the autonomic mechanism was interrupted and the adrenal cortical activity was delayed after cold, epinephrine (s.c.) or surgical operations.

Brobeck (1952) studied the neural control of ACTH secretion. They made diencephalic lesions in 25 rats of which possibly seventeen showed delayed eosinopenia after cold, epinephrine (s.c.) and laparotomy. "The series is not large enough nor are most of the lesions localised well enough to allow us to decide just what region of the diencephalon must be destroyed to prevent the autonomic response." Paraventricular nuclei were not important as after lesions of these nuclei there was usual eosinopenia after stressors. They were not required for the delayed or "metabolic" phase of the activation. Brobeck concluded that in the brain stem and the hypothalamus there was no mechanism which could increase the ACTH output in response to pain.

Harris (1944) and Green and Harris (1947) suggested that the hypothalamus could influence anterior pituitary activity by the following mechanism :—

(1) A humoral transmitter was liberated in the median eminence by nerve fibres passing from the hypothalamus to the median eminence.

(2) This humoral transmitter after being liberated in the median eminence got an entry into the portal vessels and thus got access to the anterior pituitary which was activated. This was finally elaborated by de Groot and Harris (1950) who made lesion and stimulatory experiments in the rabbit. The transverse lesions in the posterior part of the tuber cinereum or mammillary body or zona tuberalis of the pituitary abolished the response to emotional stress.



Porter (1953) made hypothalamic lesions in cats and studied eosinopenic response to epinephrine, formalin, and histamine. Lesion in the anterior hypothalamus had no effects. Posterior hypothalamic lesions blocked the eosinopenic response.

Briggs and Munson (1954) studied the anaesthetic action of morphine on hypothalamus. Morphine as such is a stressor as it leads to depletion of adrenal ascorbic acid concentration in rats, but its stressor action was abolished if the animal was first of all anaesthetised with pentobarbital sodium 40 mg./kg. 10 minutes before the morphine injection. Under these circumstances morphine could block the discharge of ACTH in response to histamine, vasopressin and laparotomy. Adrenal ascorbic acid depletion in hypophysectomized rats after ACTH administration could not be blocked by morphine. "Therefore the action of morphine must be at some point in the stimulus effector system which normally excites the ACTH secretory mechanism of the anterior pituitary. Pentobarbital was found not to be essential for the blocking effect of morphine. Unanaesthetized rats accustomed to morphine by daily administration for four days no longer responded to morphine by increased secretion of ACTH. The normal reaction of the pituitary to histamine is also blocked by morphine alone in such rats."

Munson (1954) mentioned the above findings in the discussion to Dr. Porter's paper on "The central nervous system and eosinopenia."

Similarly, deep anaesthesia with pentobarbital blocked the adrenal ascorbic acid depletion activity of salicylates when injected subcutaneously (Cronheim and Hyder, 1954).

Ohler and Sevy (1956) could inhibit the adrenal ascorbic acid depletion by proper pharmacologic agents after sham adrenalectomy, unilateral adrenalectomy, l-epinephrine, hydroamphetamine, and vasopressin. The pharmacologic agents were, adrenal cortex extract (ACE), morphine, and dibenzylamine. The above stressors have no adrenal ascorbic acid depleting effect after morphine. ACE could block the response to the operative stress and sympathomimetic amines, but it could not block the response to vasopressin. Dibenzylamine could block only the response to the sympathomimetic amines.

Stein and Mirsky (1956) supported the hypothesis that after exposure to various environmental stimuli, the hypothalamus was activated and the hypothalamic nuclei secreted vasopressin and oxytocin. These neurohormones activated the adeno-hypophysis.

Pool *et al.* (1956) studied 6 patients by implanting electrodes in the brain, anterior and ventral to the anterior commissure within 1 cm. of the midline in the region of the septal nuclei. However the electrodes were not identical in position in all patients. Stimulation of the brain near the anterior hypothalamus led to an increase in the blood and urinary steroids. Stimulation of the brain more anterior, inferior, or lateral position to those of patient no. 1 gave rise to a reduction in the steroid levels in the blood, urine and the adrenal venous blood. They suggested the possibility of activating and perhaps depressing pathways for steroid production.



Green (1956) suggested the pathway for the control of the anterior as also the posterior pituitary as :—" peripheral afferents→extra lemniscal afferent paths in the reticular activating system→lateral hypothalamus→septum→hippocampal formation→amygdala→hypothalamus (by way of the stria and medial forebrain bundle)...A second possibility is that direct relays from the septum to the amygdala may occur."

Gloor (1956) suggested that the limbic system could take part in the activation of the correlated endocrine mechanisms.

Harris (1956) summarised by saying that "the systems that we have been studying all day seem to form a hierarchy involving the effect of the external environment on the central nervous system, the pathways by which the various sensory modalities affect the hypothalamus, hippocampus, and amygdala, the anatomical pathways subserving the interplay between these and the hypothalamus, and the pathway from the hypothalamus to the posterior pituitary and the anterior pituitary glands."

From the above review it is evident that though there is no unanimity regarding the precise location of the area in the hypothalamus which can activate the ACTH release from the anterior pituitary, yet it is clear that hypothalamus produces a substance, which by its entrance into the anterior pituitary through the portal vessels, can increase the ACTH secretion.

Kovacs and Bachrach (1951) found that when an animal was exposed to the noxious stimulus, there was an antidiuretic response and also the antidiuretic substance in the hypothalamus diminished. This hypothalamic substance may come to the anterior pituitary through the pituitary portal vessels or through the systemic circulation (Hume and Wittenstein, 1950). Mirsky (1953) postulated "the metabolic responses to acute stress-inducing situations are the resultant of : (a) the secretion of ADS in the hypothalamus and the consequent activation of the autonomic nervous system, and (b) the secretion of ADS into the circulation, stimulation of the anterior pituitary gland, the secretion of ACTH and the consequent stimulation of the secretion of corticosteroids." Regarding the control of the anterior pituitary by the posterior pituitary hormones, Zuckerman (1954) concluded by saying, "if the posterior lobe hormones control the varied and varying functions of the anterior lobe, this is not yet apparent from the evidence before us"—because Nagareda and Gaunt (1951) and Stutinsky (1952) showed that **strong doses** of posterior pituitary hormone could stimulate the release of ACTH.

Scharrer and Scharrer (1954) showed that the neurosecretory fibres from the supraoptic and paraventricular nuclei were intimately connected with the blood vessels in the median eminence in birds. This was taken from the data of Assenmacher and Benoit (1953a, 1953b) and Benoit and Assenmacher (1951a, 1951b, 1951c, 1951d, 1952, 1954) and Wingstrand (1951). The peculiarity was that the neurosecretory fibres made loops in the median eminence to establish contact with the network of blood vessels which drained into the portal vessels of the anterior lobe in birds. Benoit and Assenmacher (above references) found that the portal vessels formed an indispensable link between the hypothalamus and the



anterior pituitary. In mammals, Scharrer and Scharrer (1954) stated that the vessels formed loops into the median eminence and the neurosecretory fibres terminated surrounding the loops. So a possibility remains for the neurosecretory substances to enter into the portal vessels and thus to activate the anterior pituitary. de Groot (1954) and Harris (1954) thought of this possibility. Professor Harris (1954) further said, "it is well known that stress stimuli result in posterior pituitary secretion of the antidiuretic hormone and anterior pituitary secretion of ACTH. It is dangerous though to argue from this that the mechanism regulating the secretion of these two hormones is the same. Stress stimuli will affect the activity of the endocrine system in a widespread and marked manner."

Scharrer (1954) pointed out that tuberal nuclei might send some neurosecretory substance to the anterior pituitary through the portal vessels.

White (1954) admitted that posterior pituitary hormone might have stimulating influence for ACTH release. Scharrer (1954), Shimazu *et al.* (1954), Bargmann (1954), Rothballer (1953), Bargmann *et al.* (1950), Palay (1953), Green (1951), Stutinsky (1951), Hild (1952), and Benoit and Assenmacher (1953) agreed with the possibility of the neurosecretory substance to enter into the portal system to reach the anterior pituitary.

Herlant (1954) from morphological studies in human materials came to the conclusion that the neurosecretory materials came from the median eminence towards the anterior pituitary by the pituitary-portal vessels.

Wagenvoort (1954) suggested that the neurosecretory materials came from the hypothalamus to the pituitary by the nerve fibres and the pituitary-portal vessels. Scharrer (1956) concludes, "a peptide has been obtained from the posterior pituitary which causes the release of ACTH from the anterior pituitary. There is evidence to suggest that this substance is produced, together with the other active peptides of the posterior pituitary, by the neurosecretory cells of the supraoptic and paraventricular nuclei and is released from nerve terminals into the hypothalamic-pituitary-portal vessels."

McCann and Brobeck (1954) put some evidence for the supraoptico-hypophysial system in regulating the adrenocorticotrophin secretion. They said that hypothalamic lesions which block ACTH secretion as judged by adrenal ascorbic acid depletion, adrenal weight or blood ACTH concentration in rats, destroyed a significant fraction of the supraoptico-hypophysial tract. The supraopticohypophysial tract lesion was evidenced by its location and the manifestation of diabetes insipidus. Large doses of pitressin produced ACTH secretion. Small dose of pitressin or the injection of epinephrine, histamine or pitocin did not produce significant ACTH secretion in such rats. The results indicated that the supraopticohypophysial tract may play a role in the regulation of ACTH secretion by release of antidiuretic hormone into the hypophysial portal vessels.

Okada *et al.* (1955) found some neurosecretory granules of the hypothalamohypophysial fibres to enter into the intercellular spaces of the pars tuberalis and the anterior portion of the pars distalis through the primary capillaries or their perivascular spaces. Some neurosecretory



granules from the neural lobe could enter into the caudal portion of the pars distalis through the pars intermedia.

Okada (1954) found histological changes in the anterior pituitary cells by electric stimulation of the lateral hypothalamic nucleus and the ventromedial hypothalamic nucleus. Changes opposite to each other were produced on the stimulation of these two groups of nuclei.

Shibusawa *et al.* (1954) concluded that the vagus nerve is stimulated by surgical stress. Then acetylcholine is liberated in the neurohypophysis and thereafter in the hypothalamus. Neurosecretion follows thereupon.

Shibusawa *et al.* (1955) found that after moderate doses of vasopressin there was an increase in the anterior pituitary activity for the release of ACTH in the dog, rat and man. This was evidenced by increased 17-hydroxycorticoids and eosinopenia. Thus vasopressin was thought to be an important stimulator for the pituitary-adrenocortical hormone.

Fraja and Martini (1952) found that intravenous injection of 1 U/kgm. of both oxytocic and antidiuretic hormones activated adenohypophysis to discharge ACTH, which in turn produced eosinopenia. .1 U/kgm. produced greater eosinopenia when the above two drugs were injected directly into the cerebrospinal fluid.

Bertelli and Martini (1952) found that normal rats injected intramuscularly or intravenously with .3U/rat of posterior pituitary hormones showed a fall in adrenal ascorbic acid and this fall was comparable in degree and time relationship to the fall produced by ACTH. This fall was not due to contamination as it did not occur in hypophysectomized rats.

That posterior pituitary hormones can stimulate anterior lobe activity has been shown also by Frank (1952), Gori (1953), Mirsky *et al.* (1954), Barnett (1954), and Juret and Poli-Marchetti (1953). Kovacs *et al.* (1954) reviewed the role of posterior lobe in stress responses.

Martini and Morpurgo (1955) found in rats that antidiuretic hormone produced a significant fall of the adrenal cholesterol. Oxytocin produced fall in adrenal cholesterol, but this was not significant. The same drugs injected into hypophysectomized rats did not produce any lowering of adrenal cholesterol. This demonstrated that posterior pituitary hormones were not contaminated with adrenocorticotrophic hormone to produce cholesterol depletion. "The results confirm that posterior pituitary hormones may activate the anterior lobe of the pituitary gland to discharge adrenocorticotrophic hormone and indicate that antidiuretic hormone of the posterior pituitary may be considered as a possible neurohormonal transmitting agent. Experiments to be published carried out on hypophysectomized rats bearing pituitary transplants in the anterior chamber of the eye, demonstrate that the action of posterior pituitary hormones on the adenohypophysis is direct and not mediated through nerve centres."

Arimura (1955), Itoh and Arimura (1954), and Kimura (1954) stated that under certain circumstances, posterior pituitary hormones could inhibit endogenous ACTH secretion. Mazzi (1953) found the neuro-



secretory materials in the neurosecretory fibres from the hypothalamus to the intermediate lobe but could not find the materials penetrating into the anterior lobe in amphibia.

Sobel *et al.* (1955) found in guinea pigs increased urinary excretion of corticoids after vasopressin. Manifestation of stress was not an obvious feature after increased corticoid excretion by vasopressin. Therefore it was thought to be specific. This suggested that vasopressin might be the humoral mediator of ACTH release after hypothalamic stimulation.

McDonald and Weise (1956) studied the effect of vasopressin on plasma 17-hydroxycorticosteroid levels in male and female subjects. There was a significant rise in the plasma 17-hydroxycorticosteroid during the 2 hour period of pitressin infusion and also there was a fall in the 4-hour blood eosinophil count. Similar studies carried on with highly purified arginine-vasopressin showed increased plasma 17-hydroxycorticosteroid.

Eisen and Lewis (1954) suggested that surgical stress could stimulate the hypothalamic nuclei thereby releasing vasopressin from the posterior lobe.

Hoffmann—Credner (1953) found in men that "after the flickerlight there ensues a drop in blood eosinophils despite the increased antidiuretic hormone secretion and this is ascribed to a predominance of the simultaneously produced ACTH of the anterior lobe."

Saffran and Schally (1955) studied the ACTH secretion by the rat pituitary *in vitro*. Adrenaline, nor-adrenaline, hypothalamic tissue or posterior pituitary tissue alone could not stimulate ACTH secretion. Posterior pituitary with noradrenaline caused 6 to 8 fold increase in the ACTH secretion. They concluded that posterior pituitary was possibly involved in the activation of the pituitary adrenocortical system to stress. Saffran *et al.* (1955) obtained a peptide from the posterior pituitary which stimulated the release of ACTH from the anterior pituitary.

Guillemin and Hearn (1955) studied tissue cultures of rat pituitaries. Commercial vasopressin increased the ACTH secretion from the hypothysial cells. Highly purified arginine-vasopressin has no such stimulating effect. This discrepancy in the action was due to the contamination of the commercial vasopressin by chemicals from hypothalamus.

Guillemin (1955) cultured dog and rat anterior pituitary in roller-tubes. His results demonstrated "the possibility of a humoral non-nervous control of the adeno-hypophysis by hypothalamic nuclei *in vitro*. The existence of specific hypophysiotrope hypothalamic mediator(s) is postulated. Hypothalamic control may be necessary for secretion of pituitary tropic hormones (AAF, LH), but not for secretion of pituitary trophic hormones (AWF, STH, FSH)."

Guillemin *et al.* (1956) isolated a substance from the hypothalamus which stimulated release of ACTH *in vitro*. One fraction designated as fraction D, stimulated release of ACTH *in vitro*. "After elution, only one of these components (D  $\Delta$ ) stimulated release of ACTH. Hydrolysis of fraction D  $\Delta$  revealed 17 different aminoacids; D  $\Delta$  therefore, may be a fairly complex polypeptide or still a mixture. Hypothalamic D is approximately 100 times more active than posterior pituitary D w/w."



Roberts and Keller (1955) suggested that epinephrine and cortisone activated neurohumoral pathways in the posterior hypothalamico-adenohypophysial system. Adrenaline had stimulatory effect and cortisone an inhibitory one. Moreover epinephrine could act directly on the anterior pituitary gland.

Hume (1949) prepared an extract from the hypothalamus which when injected produced good eosinopenic response in normal animals as also in animals with hypothalamic lesions which made them incapable of responding to other stressing agents tested. Slusher and Roberts (1954) isolated two purified extracts from bovine hypothalamus. These could stimulate the release of pituitary ACTH as evidenced by eosinopenia and depletion of adrenal ascorbic acid when injected intraperitoneally into intact rats. The first factor was a water soluble, nonprotein substance which could be extracted from all parts of the brain. The second factor was a lipid or lipoprotein and this was present only in the extracts of the posterior hypothalamus. This could activate only in the presence of the pituitary. "This lipoidal substance may represent in crude form the natural neurohumor presumed involved in the stress-induced release of ACTH by the adenohypophysis." Guillemin (1955) using pharmacologic blocking agents could not find any significance of adrenaline, noradrenaline, acetylcholine or histamine as specific anterior lobe activators. Harris and Fortier (1954) said that among the known biologically active compounds present in the brain, 5-hydroxytryptamine and substance P would appear worth considering.

Porter *et al.* (1955) developed a method for obtaining blood from the severed hypothalamico-hypophysial portal vessels of the heparinized dog following removal of the pituitary. The blood collected in the sella turcica from where it was aspirated out. Plasma from the carotid artery blood from the same animal served as a control.

Porter and Jones (1956) found that blood from the pituitary portal vessels contained a substance(s) which accelerated the discharge of ACTH from the anterior lobe. They further suggested that a block in the response of the rat to the stress of unilateral adrenalectomy occurred at some place other than the anterior lobe and possibly the hypothalamus. Porter and Rumsfeld (1956a) found that when a quantity of lyophilized hypothalamico-hypophysial-portal vessel plasma equivalent to 10 ml. of whole plasma was injected into hydrocortisone inhibited, intact rats, there was a decrease of  $-107 \pm 12.9$  mg. of ascorbic acid per 100 gm. of adrenal tissue. When intact rats were injected with lyophilized carotid artery plasma or when hypophysectomized rats were injected with equivalent amounts of lyophilized portal vessels plasma, there was a decrease of only  $0 \pm 2.8$  and  $-14 \pm 5.4$  respectively. The substance which was contained in the portal vessel plasma and which was responsible for the ascorbic acid reduction, was nondialyzable and following low temperature alcohol fractionation of the plasma proteins was found to be present in a single fraction. Similar activity was present in commercial pitressin. The results indicated that the active substance was either a large protein molecule or was bound to a large protein and was probably not identical with vasopressin. Porter and Rumsfeld (1956b) came to the conclusion that the



ACTH releasing activity in the portal vessel plasma was present in a globulin sub-fraction. Further purification of this sub-fraction from 10 ml. of original plasma gave a fraction which contained 1.3 mg. of protein. This caused a mean change in adrenal ascorbic acid concentration of—77 mg./100 gm. adrenal weight.

This active substance is :—

- (a) non-dialyzable—it means that it is a large protein molecule or it is firmly bound to a large molecule,
- (b) not ACTH since its activity (ascorbic acid depletion) is abolished by hypophysectomy, and
- (c) its activity is not due to epinephrine, norepinephrine or histamine because injections of large amounts of these substances into hydrocortisone inhibited intact rats do not cause any ascorbic acid depletion.

Schapiro *et al.* (1956) found a pituitary stimulating substance in the brain blood of hypophysectomized rat after electroshock stress. When brain blood from **stressed hypophysectomized rats** was injected into hypothalamic lesioned rats, there was a marked eosinopenia. The eosinopenia did not occur when brain blood from **unstressed animals** was injected into lesioned animals. Intact pituitary-adrenal axis was responsible for this eosinopenia.

The above review is about the corticotrophin releasing factor (C.R.F.) but whether the substance is same as vasopressin or a separate C.R.F. is not definitely known. The hypothalamus contains large amount of serotonin, substance P, histamine and nor-adrenaline. The concentration of these substances varies after the administration of drugs and when the nerve impulses come from the brain. Overbeek (1958) mentions "this makes it very probable that they play their part in the hypothalamic pituitary system. However, what this part is still remains to be discovered."

The histamine concentration of the anterior and posterior lobes of the pituitary, the hypothalamus and the median eminence is variable in different individuals of the same species and the concentration of histamine in these places is very high. In the median eminence the individual variation is narrower than in the anterior lobe (Harris *et al.*, 1952). They speculated that in the median eminence histamine is related to the nerve endings and it is transported to the anterior pituitary in different concentrations depending on the stage of activity. This can be more definitely proved by noting the histamine concentration of the blood from the hypophysis-portal vessels in different stress conditions and comparing it with the histamine concentration of the blood in the peripheral vein.

Histamine may reach the anterior pituitary through the general circulation. Fuche and Kahlson (1957) suggested that **physiological quantities of histamine** coming through the general circulation is an effective stimulus for increased secretion of ACTH from the adenohypophysis. The hypophysis is required for the lymphopenic response after histamine but adrenaline from adrenal medulla is not required for the lymphopenic response after histamine or emotional stimulation. The lymphopenic response to histamine was not abolished by the histamine antagonist mepyramine.



Brodish and Long (1957) report that there is a factor in the blood of hypophysectomized rats which increased the level of circulating ACTH significantly in the intact rat and that the ACTH releasing activity of the blood of the hypophysectomized animals can be decreased by pre-treatment of the animals with adrenal hormones. Eik-Nes and Brizzee (1958) have demonstrated that whole blood or blood serum from dogs completely hypophysectomized for one to five days has a factor which increases the plasma levels of 17-OHCS in the normal animal.

The following plan has been adopted in the present work :—

(1) Histamine concentration of the hypothalamus, anterior and posterior pituitary, median eminence and cerebral cortex in burns, intestinal obstruction and fracture has been noted.

(2) Histamine content of the blood from the hypophysis-portal vessels has been noted after stress and this has been compared to the histamine content of the blood from a peripheral vein.

(3) Histamine concentration in the peripheral blood of hypophysectomized and adrenalectomized dogs has been studied.

(4) Hypothalamic extracts have been prepared in different poststress days and their action on 17-OHCS secretion has been noted in the test dogs.

(5) Experiments have been done with blood, peritoneal fluid and content of fracture haematoma from different stressed conditions to note their action on the pituitary-adrenal axis.

#### Materials and methods :—

The donor animals are male dogs of 10 to 15 kg. in weight. The stressing procedures employed here are fracture of right femur, burn injury of the right hind limb by naked flame and small intestinal (low) obstruction. The animals were killed. The histamine concentration of the anterior and posterior lobe of the pituitary, hypothalamus, median eminence and cerebral cortex has been studied in the normal and stressed dogs. The blood from the hypophysis-portal vessels has been collected from the pituitary fossa after removal of the pituitary. The histamine concentration in the blood of the hypophysis-portal vessels has been compared to the histamine content in the blood of a peripheral vein and the difference has been noted. Extracts were prepared by trichloroacetic acid (Code's modification of Barsoum and Gaddum's method, 1937) and histamine has been estimated by testing the action of the extracts on the isolated terminal ileum of guineapigs.

Hypothalamic extracts have been taken from normal and stressed dogs (average of 2 dogs in each group) in different poststress days and the effect of these extracts on the adrenal venous 17-OHCS output has been noted. The extracts have been tested by injection into the carotid artery of the assay dogs and hypothalamectomized assay dogs. This route of administration is preferable as the response is very quick and a good response is noted. The extracts have been prepared with acid alcohol or trichloroacetic acid and have the properties as described by Harris *et al.* (1952).

Blood has been collected from—

(a) femoral vein of normal dogs,



- (b) right femoral vein after the right hind limb has been burned or the right femur has been fractured,
- (c) fracture haematoma,
- (d) portal vein of normal dogs and dogs with intestinal obstruction,
- (e) the pituitary fossa after the extirpation of the pituitary of normal dogs and dogs with burn, fracture and intestinal obstruction (also in different poststress days), and
- (f) jugular vein of hypophysectomized and hypophysectomized burned dogs.

The blood thus collected is injected into the carotid artery of the assay dogs and the percentage rise in 17-OHCS output in the adrenal venous blood has been noted. Peritoneal fluid collected from dogs with intestinal obstruction and .8U ACTH per kg. has been injected into the carotid artery of the assay dogs and the percentage rise in 17-OHCS output in the adrenal venous blood has been noted in each case.

Histamine concentration in the peripheral blood of hypophysectomized and adrenalectomized dogs has been noted [Code's modification of Barsoum and Gaddum's method (1937)].

The assay animals have been prepared by exteriorization of the carotid artery and the animals have been conditioned for blood removal or intra-carotid injection of blood. Cannulation of the right adrenal vein has been performed before the experiments. Hypophysectomy was done by the transtemporal route and it was considered to be complete when no pituitary tissue was detected in the pituitary fossa or in areas nearby in histological sections.

The determination of 17-hydroxycorticosteroid has been done after the method of Silber and Porter (1954).

#### RESULTS :—

##### (A)

Type of stress	Histamine concentration in $\mu\text{g./gm.}$				
	Anterior lobe of pituitary	Posterior lobe of pituitary	Hypothalamus	Median eminence	Cerebral cortex
Normal (2)	6.4	17.3	10.0	18.6	.14
Burn—1st day (2)	15.0	27.2	16.2	26.3	.32
Burn—3rd day (2)	12.2	20.1	16.1	23.5	
Fracture—1st day (2)	9.2	19.4	9.8	22.4	.19
Fracture—3rd day (2)	9.6	18.2	12.2	21.2	
Intestinal Obstruction— 3rd day (2)	12.3	22.2	12.6	22.1	.15
Hypophysectomy—1st day (2)			18.0		
Hypophysectomy and burn— 1st day (2)			20.1		
Hypophysectomy—9th day (2)			10.2		

The number in parenthesis = the number of brains taken.



(B)

Type of stress	Rise in the histamine concentration in the blood of the hypophysoportal vessels in comparison to the histamine concentration in the blood of a peripheral vein— μg./ml. blood	
Normal	.03	Average of 2 experiments in each group
Burn (1st day)	.14	
Burn (3rd day)	.12	
Fracture (1st day)	.08	
Fracture (3rd day)	.02	
Intestinal obstruction (3rd day)	.09	

(C) Experiments with hypothalamic extracts :—

Average value of 4 observations for each type of experiment has been presented.

Hypothalamic extracts from	Percentage rise in 17-OHCS output in the adrenal venous blood of the assay animals after injection of the hypothalamic extracts into the carotid artery.
(1) Normal dogs .. .. .	74.1%
(2) Burned dogs—1st day .. .. .	304%
(3) Burned dogs—3rd day .. .. .	189.2%
(4) Burned dogs—10th day .. .. .	128.2%
(5) Dogs with femur fracture—1st day .. .. .	93.8%
(6) Dogs with femur fracture—3rd day .. .. .	82.3%
(7) Dogs with small intestinal obstruction (low)—1st day .. .. .	225.5%
(8) Dogs with small intestinal obstruction (low)—3rd day .. .. .	252.2%
(9) Hypophysectomized dogs—1st day .. .. .	332%
(10) Hypophysectomized dogs—3rd day .. .. .	328%
(11) Hypophysectomized dogs—9th day .. .. .	55%
(12) Hypophysectomized burned dogs—1st day .. .. .	377%

(D) In the following experiments the action of the blood, peritoneal fluid and content of the fracture haematoma from different stress situations has been noted after injection of these substances into the carotid artery of the assay dog. Each group comprises average of 5 observations. The observations were made 20 minutes after the injection.

(i) 25 c.c. blood was taken from the femoral vein of normal dogs and injected into the carotid artery of the assay dog.

Percentage rise in 17-OHCS output in the adrenal venous blood .. .. . 8%

(ii) 25 c.c. blood was taken from the femoral vein of the particular hind limb which has been burned. It was injected into the carotid artery of the assay dog.

Percentage rise in 17-OHCS output in the adrenal venous blood.

1st day of burn .. .. .	182%
5th day of burn .. .. .	105%



(iii) 25 c.c. blood was taken from the femoral vein of the particular hind limb in which the femur has been fractured. The blood is then injected into the carotid artery of the assay dog.

Percentage rise in 17-OHCS output in the adrenal venous blood.

1st day of fracture	..	..	136%
5th day of fracture	..	..	42%

(iv) 5 c.c. blood has been aspirated from the fracture haematoma and injected into the carotid artery of the assay dog.

Percentage rise in 17-OHCS output in the adrenal venous blood .. .. .

147.5%

(v) 25 c.c. blood from the portal vein of the normal dog was injected into the carotid artery of the assay dog.

Percentage rise in 17-OHCS output in the adrenal venous blood .. .. .

10.5%

(vi) 25 c.c. blood from the portal vein of dogs with intestinal obstruction was injected into the carotid artery of the assay dog.

Percentage rise in 17-OHCS output in the adrenal venous blood .. .. .

167.7%

(vii) 5 c.c. peritoneal fluid from dogs with intestinal obstruction was injected into the carotid artery of the assay dog.

Percentage rise in 17-OHCS output in the adrenal venous blood .. .. .

111.4%

(viii) 25 c.c. blood was collected from the pituitary fossa after extirpation of the pituitary of normal dogs. The blood was injected into the carotid artery of the assay dog.

Percentage rise in 17-OHCS output in the adrenal venous blood .. .. .

185.7%

(ix) 25 c.c. blood was collected from the pituitary fossa after extirpation of the pituitary of the burned dogs. The blood was injected into the carotid artery of the assay dog.

Percentage rise in 17-OHCS output in the adrenal venous blood.

1st day of burn	..	..	..	325%
5th day of burn	..	..	..	302%

(x) 25 c.c. blood was collected from the pituitary fossa after extirpation of the pituitary of dogs with femur-fracture. The blood was injected into the carotid artery of the assay dog.

Percentage rise in 17-OHCS output in the adrenal venous blood.

1st day of fracture	..	..	..	205%
5th day of fracture	..	..	..	82%

(xi) 25 c.c. blood was collected from the pituitary fossa after extirpation of the pituitary of dogs with intestinal obstruction. The blood was injected into the carotid artery of the assay dog.

Percentage rise in 17-OHCS output in the adrenal venous blood.

1st day of intestinal obstruction	..	..	..	225%
5th day of intestinal obstruction	..	..	..	300.2%



(xii) Injection of .8 unit of ACTH per kg. into the carotid artery of the assay dog.

Percentage rise in 17-OHCS output in the adrenal venous blood .. .. . 322.3%

(xiii) 25 c.c. blood from the jugular vein of hypophysectomized dogs (3rd day of hypophysectomy) was injected into the carotid artery of the assay dog.

Percentage rise in 17-OHCS output in the adrenal venous blood .. .. . 292%

(xiv) 25 c.c. blood from the jugular vein of the hypophysectomized burned dogs (3rd day of hypophysectomy) was injected into the carotid artery of the assay dog.

Percentage rise in 17-OHCS output in the adrenal venous blood.  
1st day of burn .. .. . 318%

(xv) 25 c.c. blood was drawn from the right femoral vein of the hypophysectomized (3rd day) dogs with burn injury of the right hind limb. The blood was injected into the carotid artery of the assay dog.

Percentage rise in 17-OHCS output in the adrenal venous blood .. .. . 288%

(xvi) 25 c.c. blood was drawn from the jugular vein of the hypophysectomized (9th day of hypophysectomy) dogs. The blood was injected into the carotid artery of the assay dog.

Percentage rise in 17-OHCS output in the adrenal venous blood .. .. . 51.2%

(xvii) The above experiments were repeated (except exp. no. xii) and 17-OHCS output in the adrenal venous blood in hypophysectomized assay dogs was noted. The presence of the hypophysis in the assay dogs is required for good response.

(A) The histamine concentration in the hypothalamus, median eminence and in the anterior and posterior lobe of the pituitary of the dog is high. This corroborates the findings of Harris *et al.* (1952). During stress, the histamine concentration in the above areas of the brain rises more and fluctuates in poststress days. The maximum concentration is found in the median eminence region and the minimum is found in the cerebral cortex. In stress, there is rise of histamine concentration in the cerebral cortex also. The maximum concentration of histamine is found in burn in different areas of the brain and the pituitary. In the hypophysectomized dogs the histamine concentration in the hypothalamus is very high and highest level is found in such an animal after burn trauma. The value comes down to a low level on the ninth day of hypophysectomy. Histamine concentration in the hypothalamus, median eminence and pituitary of dogs diminishes on cortisone treatment. The concentration is high in the adrenalectomized dogs.

(B) Blood in the hypophysiportal vessels contains more histamine in comparison to the histamine content of the blood in the peripheral vein. The rise is more in stress and the maximum rise is found in burn. In poststress days the difference is not so much as that found in the first



day of stress but in burn it is still higher than that found in the normal animal. During stress the histamine content of the blood in the peripheral vein is high. Histamine concentration in the blood of the hypophysiportal vessels is low in dogs treated with cortisone.

(C) In the next experimental group, the percentage rise in 17-OHCS output in the adrenal venous blood of the assay dogs has been noted after intracarotid injection of the hypothalamic extracts. The hypothalamic extracts have been taken from normal, stressed, hypophysectomized and hypophysectomized stressed dogs. The hypothalamic extracts have been taken also in different poststress days. Hypothalamic extracts taken from normal dogs and injected into the carotid artery of the assay dogs could elevate 17-OHCS output in the adrenal vein within 20 minutes of injection. With the injection of the hypothalamic extracts from dogs with burn, fracture and intestinal obstruction, the percentage rise in 17-OHCS output in the adrenal venous blood was good and it was better than that found after injection of the hypothalamic extracts from normal dogs. The maximum response was found after the injection of the hypothalamic extracts from burned dogs (1st day of burn). The percentage rise in 17-OHCS output in the test dogs varied when the hypothalamic extracts from dogs in different poststress days were tested. Good response was achieved when the hypothalamic extracts were taken from hypophysectomized dogs (1st and 3rd day of hypophysectomy) and injected into the carotid artery of the test dogs. Burn trauma applied to hypophysectomized dogs (1st day of hypophysectomy) could increase the potency of the hypothalamic extracts. When the hypothalamic extracts were taken from hypophysectomized dogs on the ninth day of hypophysectomy and injected into the assay dogs, the response was lower than that found after injection of the hypothalamic extracts from normal dogs. With cortisone treatment in the hypophysectomized dogs, there is lowered potency of the hypothalamic extracts when they are tested in the assay dogs. Extracts from the cerebral cortex could increase 17-OHCS output but the magnitude of response was lesser than that found after injection of the hypothalamic extracts.

The presence of the hypophysis was required in the assay animals for the response after injection of the extracts. Anaesthesia with pentobarbital sodium and hypothalamectomy could not block the response.

(D) There was 8% rise in 17-OHCS output in the adrenal venous blood when blood from the femoral vein of a normal dog was injected into the carotid artery of the assay dog. The rise is more when the blood is taken from the femoral vein of the particular hind limb which has been burned or fractured and it is injected into the assay dog. The blood from the fracture haematoma and the peritoneal fluid during intestinal obstruction contains a factor which can raise 17-OHCS output in the adrenal venous blood of the assay dog. The blood from the portal vein during intestinal obstruction also contains such a factor. The blood from the portal vein and peripheral vein of a normal dog does not contain it. There was 185.7% rise in 17-OHCS output in the adrenal venous blood of the assay dog when blood was collected from the pituitary fossa after extirpation of the pituitary of a normal dog and injected into the



carotid artery of the assay dog. Blood similarly collected from the stressed dogs and injected into the assay dogs could increase 17-OHCS output in the adrenal venous blood of the latter. There was 325% rise when blood was collected from the pituitary fossa on the first day of burn and tested. It was 302% on the fifth day of burn. With fracture, the value was 205% on the first day and 82% on the fifth day. It was 225% on the first day of intestinal obstruction and there was a further rise to 300.2% on the fifth day. There was 322.3% rise in 17-OHCS output in the adrenal venous blood after injection of ACTH (.8 U/kg.) into the carotid artery of the assay dog. The percentage rise was good when blood was collected from the jugular vein of hypophysectomized and hypophysectomized burned dogs (burn trauma on the third day of hypophysectomy) and injected into the carotid artery of the assay dog. On the ninth day of hypophysectomy only 51.2% rise was noted. There was 288% rise in 17-OHCS output, when 25 c.c. blood from the right femoral vein of the hypophysectomized (3rd day) dog burned on the right hind limb, was injected into the carotid artery of the assay dog. This percentage rise is identical to that seen after injection of jugular venous blood from hypophysectomized dog (3rd day). With cortisone treatment in the hypophysectomized dogs, there is lowered potency of the jugular venous blood when it is tested in the assay dogs.

The presence of the hypophysis is required for the responses except experiment no. xii. Anaesthesia with pentobarbital sodium cannot block the responses.

#### (E) Histamine in the peripheral blood of hypophysectomized and adrenalectomized dogs :—

Histamine concentration in the peripheral blood of hypophysectomized dogs and hypophysectomized burned dogs is high. It rises after hypophysectomy and soon attains high level within the first week. Tane *et al.* (1958) found that histamine level in blood was high within the first week of hypophysectomy in rabbits.

In the adrenalectomized dogs, there is rise in the histamine concentration in the peripheral blood. With cortisone treatment in such dogs, there is a reduction in the concentration of histamine in blood. This has been observed also by Code and Mitchell (1957) in dogs and by Tane *et al.* (1958) in rabbits. Adrenocortical extract inactivates histamine in animals (Perla and Marmorston-Gottesman, 1931 ; Ingle, 1937 ; Rose and Karady, 1939 ; Rose, 1939 ; Wilson, 1941). Ungar (1944) stated that the inhibitory substance of histamine liberation present in active sera was produced by the pituitary and acted through the adrenals. Thus the adrenocortical extract either destroys histamine in the blood or inhibits the liberation of the same from the tissues.

After hypophysectomy, the atrophic adrenal glands retained a certain degree of function (Noble and Collip, 1941). Pickford and Vogt (1951) state that complete hypophysectomy does not abolish the secretion of cortical hormone by the dog's adrenal. So the atrophic adrenal glands after hypophysectomy can regulate the histamine metabolism.



## DISCUSSION

The hypothalamus, median eminence and the anterior and posterior lobe of the pituitary gland of the dog have got high concentration of histamine. The concentration varies depending on the type and duration of the stress. Very high concentration of histamine is found in burn. It is also high in the hypothalamus of hypophysectomized (1st day) dogs and hypophysectomized burned dogs.

The hypothalamic extracts were tested in the assay dogs by injecting the substances into the carotid artery and noting the percentage rise in 17-OHCS output in the adrenal venous blood. The percentage rise was good when hypothalamic extracts from stressed dogs were tested. The response varied in different poststress days depending on the type and duration of the stress. Hypothalamic extracts from hypophysectomized burned dogs gave the best response. Hypothalamic extracts from hypophysectomized dogs on the first and third day of hypophysectomy gave good response but the hypothalamic extracts from hypophysectomized dogs on the ninth day of hypophysectomy did not give good response. The difference in response may be due to the following facts :—

(a) On the first and third day of hypophysectomy, the animal is in stressed condition which has given rise to increased production of histamine in the hypothalamus ; but on the ninth day, the intensity of stress has passed off and so the histamine content has decreased. This has been actually demonstrated by noting the histamine concentration of the hypothalamus of the hypophysectomized dogs on the first, third and ninth day of hypophysectomy.

(b) After hypophysectomy, histamine cannot come from the hypothalamus and median eminence region to the hypophysis because of its absence. So the level of histamine is high in the hypothalamus in immediate posthypophysectomy days. Subsequently, with the new formation of vascular communication, the raised concentration of histamine comes down to a low level.

The presence of the hypophysis is required for the response in the assay animals. Fuche and Kahlson (1957) stated that hypophysis is required for the lymphopenic responses after histamine. Nasmyth (1948) showed that histamine in the hypophysectomized rats does not give rise to an appreciable fall in adrenal ascorbic acid. Vogt (1951) came to the conclusion that the action of histamine on the adrenal cortex is mainly due to the release of ACTH. The direct action of histamine on the adeno-hypophysis can also be seen in the experiments of Gray and Munson (1951). They found that rats pretreated with cortical steroids did not show fall in adrenal ascorbic acid after histamine. Cortical steroids are known to depress the adeno-hypophysial activity after different types of stimuli.

Histamine is transported through the hypophysioportal vessels to the anterior pituitary. The blood of the hypophysioportal vessels contains



more histamine than the blood in the peripheral vein and during stress there is further rise in the histamine content of the blood of the hypophysiportal vessels. The histamine content of the anterior lobe of the pituitary is high. As the histamine content of the posterior lobes of the pituitary is also high, there is a possibility that histamine may be transported from the posterior to the anterior lobe. Thus the anterior lobe of the pituitary is having a good supply of histamine from three sources :—

- (a) through the general circulation
- (b) through the hypophysiportal vessels
- (c) from the posterior to the anterior pituitary.

The high level of histamine in the anterior pituitary is a good stimulant for the anterior hypophyseal cells to discharge more ACTH during stress.

It has been found that there is 185.7% rise in 17-OHCS output in the adrenal venous blood of the assay dogs, when blood has been collected from the pituitary fossa after extirpation of the pituitary of a normal dog and the blood is injected into the carotid artery of the assay dogs. The rise is more when blood has been similarly collected from stressed dogs and tested. The response is variable when blood is collected from stressed dogs in different poststress days depending on the type and duration of the stress. The presence of the pituitary is required in the assay dogs for the response.

Porter and Jones (1956) suggest that blood from the hypophyseal portal vessels contains a substance(s) which accelerates the discharge of ACTH from the anterior lobe of the pituitary. Porter and Jones (1956) further mention that "the active substance is either a large protein molecule or is bound to a large protein and is probably not identical with vasopressin." In the present investigation, it has been found that histamine may be one such active substance present in the blood of the hypophysiportal vessels and it can stimulate the discharge of ACTH from the adenohypophysis.

Blood in the femoral vein of a normal dog does not contain a substance which can increase the adrenal 17-OHCS output when injected into the carotid artery of the assay dog. Blood from the femoral vein of the hind limb which is burned or injured contains such a substance. The concentration of the substance varies in poststress days. It has been mentioned before that histamine is increased in the blood of the peripheral vein during stress. So there is a possibility that histamine is such a substance contained in the blood during stress and it can stimulate the anterior pituitary to discharge more ACTH and this can increase 17-OHCS output. Blood from the fracture haematoma, blood from the portal vein during intestinal obstruction and peritoneal fluid from dogs with intestinal obstruction contain this substance (histamine).

Jugular venous blood of the hypophysectomized dogs and hypophysectomized burned dogs contains this substance when the blood is taken on the third day of hypophysectomy. Blood from the femoral vein of the hypophysectomized burned dog is very rich in histamine. On the ninth day of hypophysectomy, the jugular venous blood does not contain



the substance so much as is found on the third day of hypophysectomy. Eik-Nes and Brizzee (1958) stated that whole blood and blood serum from dogs completely hypophysectomized for one to five days contain a factor which will increase the plasma levels of 17-OHCS in the normal dog. Brodish and Long (1957) found that the ACTH releasing substance in the blood of the hypophysectomized animals could be decreased by treatment with adrenal hormones. Vogt (1951) studied the cortical secretion of the isolated perfused adrenal by noting the rate of cortical secretion per min. per g. adrenal [expressed as weight of adrenal tissue ('g. gland') containing the same activity]. She found the mean value to be 6.5, when the isolated dog's gland was perfused with blood from normal dog. The mean value was 7.1 when the isolated dog's gland was perfused with blood from hypophysectomized dog. The highest yield 10.0 was seen in experiment number 261 where the perfusing blood was taken from the dog hypophysectomized 8 weeks before use and completely lacking in pars tuberalis. In the other two experiments (experiment numbers 212 and 210), where the blood for perfusion has been taken from hypophysectomized dogs, the values are 3.4 and 8.0 respectively. Thus a varied response is noted with the blood of the hypophysectomized donors. We have stated before that histamine concentration in the blood rises in the hypophysectomized dogs. So the substance contained in the blood of the hypophysectomized dogs may be histamine and this is responsible for the increase in the activity of the pituitary-adrenal-axis of normal dogs when injected into them. In the hypophysectomized dogs, the production and concentration of histamine in the hypothalamus is maximum. This helps in the increase of the histamine concentration of the blood. Moreover in the first week after hypophysectomy, the low level of the adrenocortical steroids in the blood helps in the rise of the histamine concentration. Subsequently some adjustment in the histamine metabolism occurs. On the ninth day of hypophysectomy, the concentration of histamine in the hypothalamus is low and also it is known that atrophic adrenal cortex after hypophysectomy can regulate the histamine metabolism. So on the ninth day of hypophysectomy, the jugular venous blood does not contain the substance to a great extent. Moreover, the stress of hypophysectomy is not so much on the ninth day of hypophysectomy. However, Vogt found increased cortical secretion when she used blood from hypophysectomized dog two months after the operation. Possibly there is increased histamine concentration in the blood and she states that histamine stimulates the adrenal cortex in some experiments. Though "under natural conditions, however, the amount of histamine likely to occur in the blood will be too small to enhance cortical secretion except by indirect means which lead to a release of ACTH."

**Central control of ACTH discharge by histamine :—**A type of mechanism exists for the discharge of ACTH by histamine coming from the median eminence and the hypothalamus. Increased level of corticoids inhibits the formation of histamine or helps in the destruction of it in the areas of the brain mentioned above and so less histamine comes to the anterior pituitary through the hypophyseal portal vessels and less ACTH is secreted. Thus cortical steroids are less in circulation and so again histamine is released, which then, stimulates adeno-hypophysial ACTH



discharge. It has been stated before that histamine concentration in the hypothalamus of hypophysectomized animals is very high. This is because of the lowered level of corticosteroids in the blood of such animals. It has been also observed that histamine concentration of the hypothalamus, median eminence and pituitary of dogs diminishes on cortisone treatment. The concentration is high in the adrenalectomized dogs. With cortisone treatment in the hypophysectomized dogs, there is lowered potency of the hypothalamic extracts when they are tested in the assay dogs. Histamine concentration in the blood of the hypophysiportal vessels is low in cortisone treated dogs. In stress situations, however, it is found that there is high level of corticoids in the adrenal venous effluent and also the histamine concentration in the hypothalamus and median eminence is high.

This can be explained as follows :—

The continuous overproduction of histamine in the brain during stress cannot be completely blocked by the increased level of corticosteroids in blood.

### SUMMARY

In the present investigation, it has been noted that histamine carried by the blood in the hypophysiportal vessels is a good stimulant for the anterior pituitary to discharge more ACTH, which in turn, increases 17-OHCS output from the adrenal gland during stress. Blood in the hypophysiportal vessels contains more histamine than the blood in the peripheral vein. Histamine carried also by the systemic circulation is a good stimulant for the discharge of ACTH from the anterior pituitary during stress. Histamine concentration in the hypothalamus, median eminence, and anterior and posterior lobe of the pituitary of the normal dog is high. Higher level of histamine in the above areas and also in the cerebral cortex is found in stress. The level fluctuates in post-stress days depending on the type and duration of stress. Histamine concentration in the hypothalamus of the hypophysectomized dogs is high. In stress, in such an animal, there is further rise in the concentration of histamine. On the ninth day of hypophysectomy, histamine concentration in the hypothalamus is low.

Hypothalamic extracts from stressed dogs can increase 17-OHCS output in the adrenal venous blood of the assay animals when injected into the carotid artery. The magnitude of response varies depending on the type and duration of stress. Hypothalamic extracts from hypophysectomized dogs on the first and third day of hypophysectomy give good response. The response is not good when the hypothalamic extract is taken from the hypophysectomized dogs on the ninth day of hypophysectomy. The hypothalamic extract from the hypophysectomized burned dog gives the



best response. Extracts from cerebral cortex can increase 17-OHCS output but the magnitude of response is lesser than that after injection of hypothalamic extracts.

Blood in the peripheral vein of a normal dog does not contain a substance which can raise the adrenal 17-OHCS output much in the assay dogs, when the blood is injected into the carotid artery. Blood from the peripheral vein of burned or injured dogs contains it. Blood from the fracture haematoma, peritoneal fluid from dogs with intestinal obstruction and blood from the portal vein of dogs with intestinal obstruction also contain the substance.

Blood collected from the pituitary fossa after extirpation of the pituitary and injected into the carotid artery of the assay dog can increase 17-OHCS output. Blood similarly collected from stressed dogs and tested gives better response. In poststress days the response varies.

Jugular venous blood from hypophysectomized (3rd day) and hypophysectomized burned dog (3rd day of hypophysectomy and burned) gives good response when tested in the assay dogs. Blood from the right femoral vein of the hypophysectomized (3rd day) dogs with burn injury of the right hind limb, gives good response. Jugular venous blood from hypophysectomized dogs on the ninth day of hypophysectomy does not give good response.

Histamine concentration in the peripheral blood of hypophysectomized and hypophysectomized burned dog is high. In the adrenalectomized dogs, histamine concentration in the peripheral blood is also high. With cortisone treatment in such dogs, the level of histamine comes down.

A type of central control of ACTH discharge by histamine has been stated.



## CHAPTER VII

### FRACTURE, BURN AND CHANGES IN THE NEUROSECRETORY SUBSTANCE

The presence of posterior lobe hormones in the hypothalamus was shown by Abel (1924), Sato (1928), Trendelenburg (1928), and Melville and Hare (1945). In the hypothalamus of many types of vertebrates and man, cells with cytological characteristics of glandular structures were described by E. Scharrer (1928, 1930, 1932, 1933, 1934, 1935, 1941, 1951, 1952, 1954), Scharrer and Gaupp (1933), Scharrer *et al.* (1945), Scharrer and Scharrer (1937, 1940, 1945, 1954), Scharrer and Wittenstein (1952). The posterior lobe hormones were shown to be originated from the neurosecretory cells of the hypothalamus by Hild and Zetler (1951a, 1951b, 1952, 1953a, 1953b), Ortmann (1950, 1951), Zetler (1952, 1953a, 1953b), Bargmann (1949a, 1949b, 1953, 1954), Bargmann and Hild (1949), Bargmann and Scharrer (1951), Sloper and Adams (1956).

Vogt (1953) thought that the hypothalamic nuclei produced vasopressin and the posterior lobe gave rise to oxytocin.

It is not only the supraoptic and paraventricular nuclei which produce neurosecretions but Smith (1951) said that the mammilloinfundibular nuclei of mammals should also be considered. E. Scharrer (1954) suggested, "it could be that the tuber nuclei indeed produce a material that we cannot stain and that they produce the factor that Dr. Harris has postulated as the one that is carried by the portal vessels to the anterior lobe. In this case we would have another type of neurosecretory cell for which we have as yet no stainable material."

As to the nature of the neurosecretory substance Schiebeler (1952a, 1952b) and Hild and Zetler (1952) thought that it was a carrier for the hormones and was of glyco-lipo-protein character. Barnett (1954) accepted this view and he also showed "that the bisulfidepositive material was depleted from the neurohypophysis of rats after dehydration and reaccumulated on rehydration and that it was depleted upon stress."

Adams and Sloper (1955) developed a histochemical technique involving reduction of ferric-ferricyanide and it reacted only with high concentration of cystine "and there is some 19% of cystine in Du Vigneaud's Octa-peptides. Consequently this technique has the advantage of demonstrating neurosecretory material but little else in the hypothalamus." They thought that it was the hormone itself. They demonstrated this bright blue neurosecretory substance in the neurohypophysis and the supraoptic nuclei of man and animals. They also were in favour of the view that the posterior lobe hormones were produced in the hypothalamus.

The neurosecretory substances passed from the hypothalamus to the pituitary along the axons. Scharrer (1956) concluded, "a peptide has been obtained from the posterior pituitary which causes the release of



ACTH from the anterior pituitary. There is evidence to suggest that this substance is produced, together with other active peptides of the posterior pituitary, by the neurosecretory cells of the supraoptic and paraventricular nuclei and is released from nerve terminals into the hypothalamic-pituitary-portal vessels."

Hild (1956) found that bioassays of posterior pituitary tissue which was grown *in vitro* did not show the evidence of production of the hormones. Within 7 to 10 days, the hormones contained in the tissues are inactivated. Neurosecretory cells which were grown *in vitro* for from 12 to 68 days had small amount of stainable neurosecretory substance.

Neurosecretory substances could be traced from the nerve cells in the hypothalamus to the infundibulum along the axons. This was shown by Scharrer and Scharrer (1944), Palay (1945), Bargmann (1949a, 1949b). The material might be carried by the axoplasm current and Palay (1945) supported Weiss-theory of axoplasmic migration (Weiss, 1944). Passage of neurosecretory substances from hypothalamus to the posterior pituitary was shown by Hild (1951, 1953), Stutinsky (1952, 1954), Mazzi (1954), Benoit and Assenmacher (1954), Hild and Zetler (1953), Scharrer and Wittenstein (1952), Drager (1950). Wagenvoort (1954) and Sloper (1954) found that after hypophysectomy in the rat, cat and dog, Gomori-positive granules were stored in the hypothalamic nuclei and fibres. This indicated that as the neurosecretory substances could not reach the posterior pituitary because of its absence, they were stored in the hypothalamus and fibres.

In severely dehydrated animals, the nerve endings of the supraoptico-hypophysial tracts in the neural lobe did not contain neurosecretory granules (Ortmann, 1950, 1951; Hild, 1951; Hild and Zetler, 1953; Leveque and Scharrer, 1953). The antidiuretic hormone content of the neurohypophysis in dehydrated animals was lower than that in animals with water balance (Kovacs and Bachrach, 1951; Hild and Zetler, 1952). 5-7 days after neurohypophysectomy ADH could be found in the blood stream (Lloyd *et al.*, 1954; Lloyd and Pierog, 1955; Mirsky, Stein and Paulisch, 1954).

Billenstein and Leveque (1955) studied the reorganization of the neurohypophysial stalk following hypophysectomy in the rat. The study consisted of the examination of the proximal portion of the cut pituitary stalk in the totally hypophysectomized rats from 3 days to 5½ months after the operation.

### 3 days after hypophysectomy :—

Haemorrhage was marked and there was exudate. Pituicytes in various stages of mitosis were found. Neurosecretory material was present in the center of the stalk.

### At 5 days :—

Haemorrhage and exudate disappeared. Mitotic figures in the pituicytes were absent. Vascular reorganization was seen. Neurosecretory material was still prominent. This material also surrounded a few blood vessels.



## 2 weeks after hypophysectomy :—

There was paucity of neurosecretory material. Only scattered granules were found. The whole area appeared to be much vascular. The proximal cut end of the stalk seemed to be rounded off. The diminished amount of neurosecretory material might be due to :—

(a) its entry into the blood vessels and,

(b) "many of these cells are in the process of recovering from the chromatolytic effects of axon severance and therefore are unable to synthesize posterior lobe hormones."

## 2 months after hypophysectomy :—

There was considerable reaccumulation of neurosecretory substance. This accumulation started from the third week. "In general, the reorganised end of the stalk has the histological appearance of a normal neural lobe."

## At 5½ months :—

The cut end of the stalk increased in width and a storage depot for the posterior lobe hormone was reorganized. There was increased accumulation of neurosecretory material round about the portal vessels in comparison to the unoperated animals. Barnett (1954) found increased disulfide groups in the infundibular stalk in the hypophysectomized rat. Lloyd and Pierog (1955) found increased ADH content in the median eminence of the hypophysectomized rat.

Billenstien and Leveque (1955) also studied the functional competency of the reorganized neurohypophysis. They said, "by our methods mobilization of the neurosecretory substance from the reconstituted structure could not be detected when dehydration procedures were used alone. However, the administration of adrenal cortical substitution therapy plus dehydration caused the reorganized neurohypophysis to be depleted of neurosecretory material. Thus, morphological evidence is presented to the effect that the reorganized neurohypophysis could be a possible source of the antidiuretic substance found in the blood of the hypophysectomized rat by other investigators."

Rothballer (1953) found a characteristic response taking place in 3 stages after needle-prick in rats. Within one to two minutes after stimulation there was vasodilatation and the neurosecretory material moved towards the lumen of the blood vessels. Within 4-6 minutes, the vasodilatation was too much with loss of neurosecretory material. From 1 to 3 hours, the restoration process started and was completed. The neurosecretory substances were present in the blood vessels as tiny flecks of material staining deep blue in the vessels amongst the erythrocytes or hanging into the lumen from the vessel wall.

Rioch (1938) found intravascular colloid in this region. Hanström (1952) showed confirmatively intravascular discharge of N.S. substance. Scharrer and Frandson (1954) thought that under normal conditions the neurosecretory substance also passed through the walls of the vessels in



granular form presumably to be dissolved in the blood in the neurohypophysis of dogs.

Rothballer (1953) considered that the same stimulus releasing the neurosecretory material gave rise to the discharge of ACTH and it was said that "these may be two parallel phenomena in the organism's response to noxious stimuli, but the possibility that they are more closely related deserves attention."

In this portion of my work I intend to study the discharge of neurosecretory substance from the hypothalamic cells and the posterior pituitary after simple fracture of femur and burn. These have been studied in the normal, spinal transected and hypophysial stalk-sectioned animals.

#### Materials and methods of the work :—

Male guineapigs for short term experiments and dogs for spinal transection and pituitary stalk section experiments have been used. Guinea pigs were of 300-450 gm. in weight and they received ascorbic acid (5 mg. per 100 gm. of body weight) by subcutaneous injection once a day for 5 days before the experiment. Male dogs were of 10-15 kg. in weight.

The operations have been performed under ether or pentobarbital anaesthesia. Drinking water was given freely to the animals. Psychological tension is diminished by bringing the animals daily to the experimental room for a few days and handled before actual experiment was performed. Spinal transected animals have been used for this experiment after one month. The hypophysial stalk-sectioned animals mean that the stalks have been cut and measures taken to prevent regeneration of vessels and these animals have been used at the end of two weeks and one month. The tissues (brain and pituitary) have been fixed in Bouin's fixative and the staining procedure is chromealum-haematoxylin and phloxine method of Gomori.

#### Findings :—

In the normal guineapigs and dogs, the neurosecretory substance is found in the supraoptic and paraventricular nuclei and along the nerve tracts into the posterior pituitary. The neurosecretory substance is also seen in extraneuronal areas.

After fracture and burn in the normal animals there is loss of neurosecretory substance from the hypothalamic nuclei within 5 minutes with vascular congestion taking place not only within the neurohypophysis but also in the hypothalamic area. The neurosecretory substance is seen in the vessels of the neurohypophysis and also in the vessels of the stalk region of the pituitary and the primary capillary plexus of the portal vessels. The neurosecretory substance can also be demonstrated in the anterior pituitary. 3 hours after stress the store of neurosecretory substance in the hypothalamic nuclei is seen to be starting replenishment.

In the spinal transected animals there is loss of neurosecretory substance from the hypothalamic nuclei after stress.



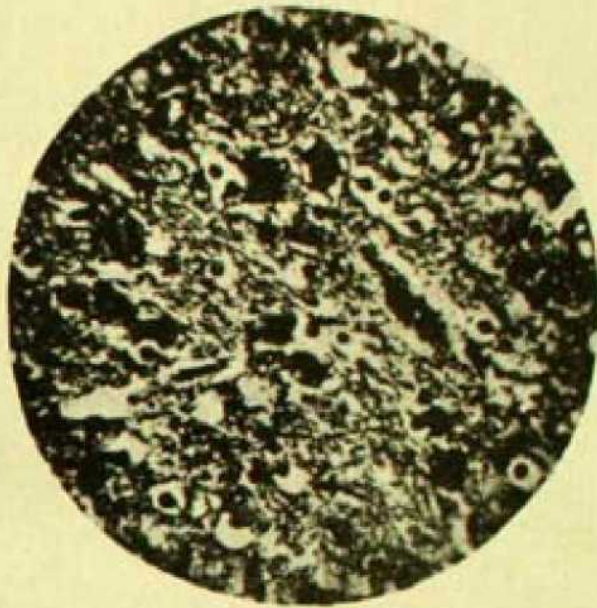


Fig. 14.—Anterior hypothalamic nuclei in a normal guinea-pig showing neurosecretory substance.

(Gomori's CAHP stain ; magnification  $\times 450$ )

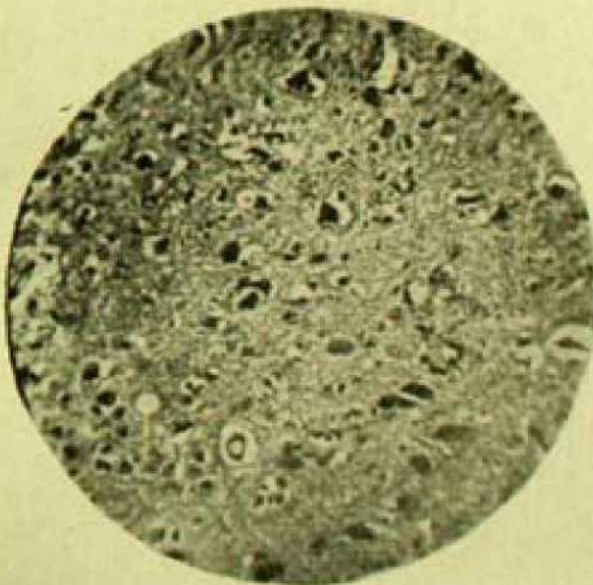


Fig. 15.—Depletion of the neurosecretory substance after fracture. (Gomori's CAHP stain ; magnification  $\times 215$ )



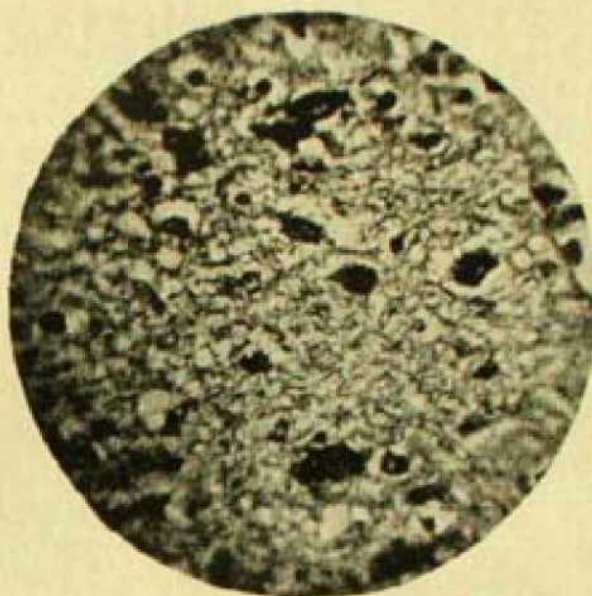


Fig. 16.—Filling up of the nuclei with neurosecretory substance after fracture.  
(Gomori's CAHP stain ; magnification  $\times 450$ )

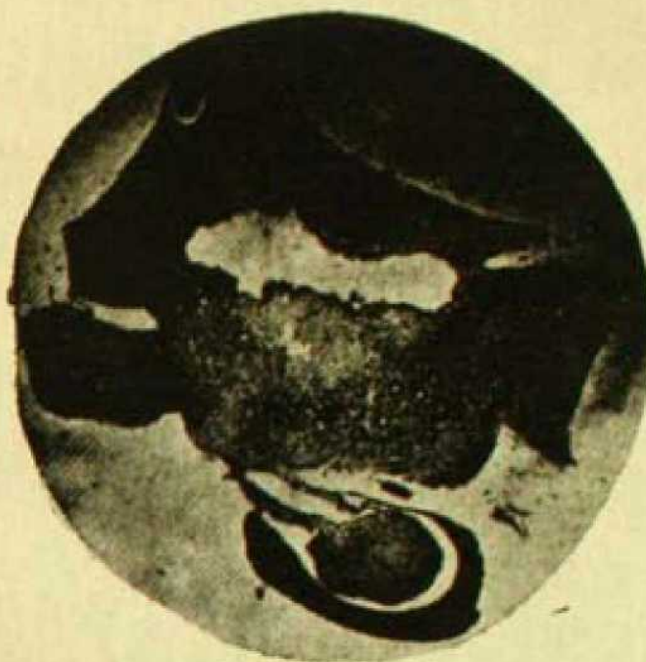


Fig. 17.—Microphotograph showing hypothalamus, stalk and pituitary (dog).  
(Gomori's CAHP stain)

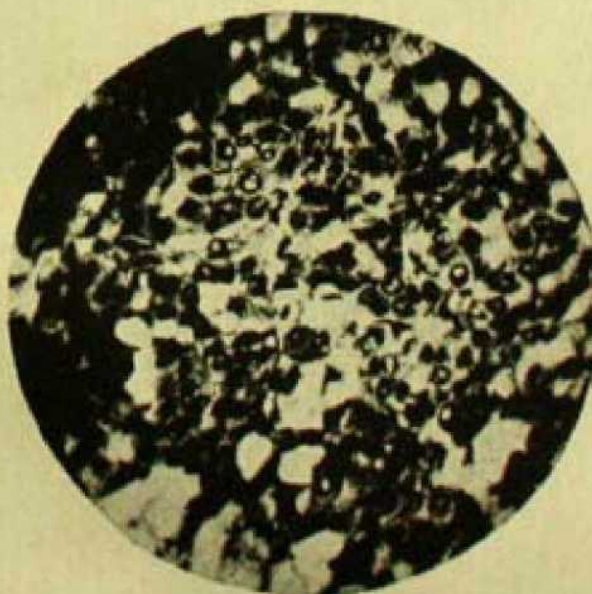


Fig. 18.—Neurosecretory substance in the anterior pituitary (dog) after fracture.  
(Gomori's CAHP stain ; magnification  $\times 450$ ).

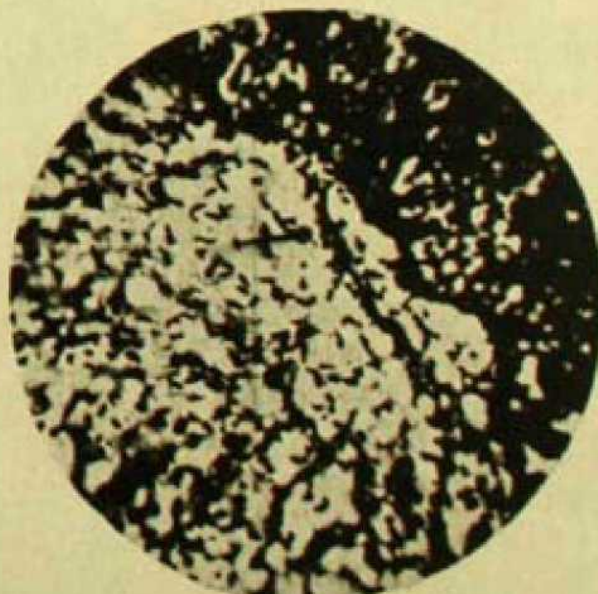


Fig. 19.—Microphotograph showing neurosecretory substance in the margin of the posterior pituitary (dog) after fracture.  
(Gomori's CAHP stain ; magnification  $\times 450$ ).





Fig. 20.—Anterior hypothalamic nuclei of the dog showing depletion of the neurosecretory substance after burn.  
(Gomori's CAHP stain ; magnification  $\times 450$ ).

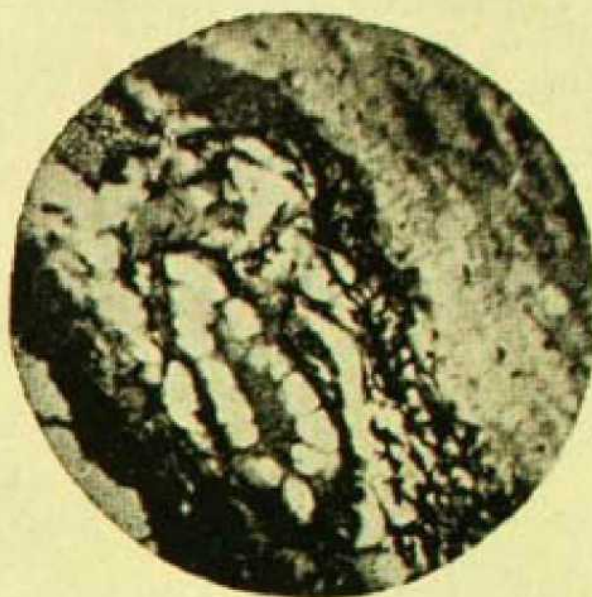


Fig. 21.—Neurosecretory substance in the primary capillary plexus of the dog after fracture.  
(Gomori's CAHP stain ; magnification  $\times 450$ ).

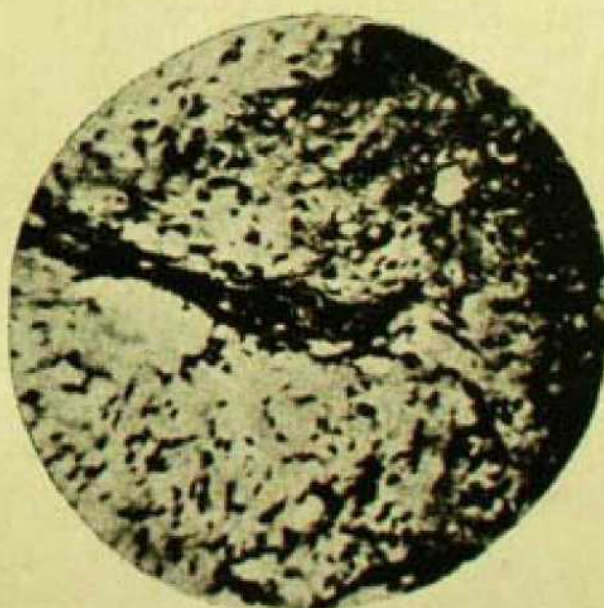


Fig. 22.—Neurosecretory substance in the form of flecks in a blood vessel of the posterior pituitary of the dog after fracture.  
(Gomori's CAHP stain ; magnification  $\times 450$ ).



### Pituitary stalk-sectioned animals :—

#### 2 weeks after stalk section :—

The neurosecretory substance in the proximal stump is lesser than that found after one month. The neurosecretory substance in the hypothalamic nuclei also shows similar feature. In the immediate post-operative period the neurosecretory substance is in excess.

After stress the loss of neurosecretory substance both from the stump as well as from the nuclei is found.

#### 1 month after stalk section :—

The neurosecretory substance in the proximal stump of the cut stalk is found to be in excess, and a similar feature is found in the hypothalamic nuclei. After stress, the substance is diminished both in the stump and also in the nuclei.

### Discussion :—

After stress in the normal animals, discharge of the neurosecretory substance from the hypothalamic nuclei and its entry into the hypophyseal portal vessels occurs. This substance has been found in the anterior pituitary suggesting that this may stimulate the anterior pituitary during stress. This corroborates Scharrer's (1956) view.

In these animals the psychological tension which may lead to stimulation of the hypothalamus has been curtailed by familiarity and also the afferent impulses coming from the extremities via the spinal cord have been cut by the spinal transection. Still in these animals we find discharge of neurosecretory substance from the hypothalamic nuclei after stress. This can be explained by the humoral mechanism affecting the nuclei as it has been mentioned by Scharrer and Scharrer (1954) that "the nuclei supra-opticus and paraventricularies possess an exceedingly dense capillary bed ; the neurosecretory cells are intimately associated with the blood capillaries. This rich vascularization is understandable since the cells might be expected to have a high metabolic rate and to require more nutrient and oxygen than other nervous elements." The pituitary stalk-sectioned animals show more or less the same features as described by Billenstein and Leveque (1955). After stress in these animals there is a depletion of the neurosecretory substance from the proximal stump of the cut stalk and this suggests that it enters into the systemic circulation. However, after stress, excess of this substance may act on the anterior pituitary coming through the systemic circulation and stimulates ACTH release.

### Conclusion :—

Discharge of neurosecretory substance after stress on normal, spinal transected and pituitary stalk-sectioned animals has been noted and there is a possibility that this substance has a role in adenohypophyseal ACTH discharge during stress.



## CHAPTER VIII

### ADRENOCORTICAL FAILURE IN BURN AND PATHWAYS REQUIRED IN THIS CONDITION FOR SECRETION OF ACTH AND ADRENOCORTICAL HORMONES

Sevitt (1957) discussed the problem of adrenocortical failure in burns. He mentioned that acute adrenal failure might occur either at the adrenal level or at the hypothalamic-pituitary level.

From my observations (Roy, 1953) it is found that failure of hypophysioadrenocortical response to stimulant (1% potassium chloride) may occur in burn. 1% potassium chloride 3 c.c. was injected into the carotid artery and intracranial venous effluent was collected from the jugular vein. 1 c.c. plasma from this blood when injected into the adrenal artery did not show much increase in the bio or chemocorticoid content of the adrenal venous effluent when compared to the corticoid content of the adrenal venous effluent in the normal dog (compare experiment number 15 with experiment number 21). In the burned dog the adrenal venous corticosteroid content was high suggesting that the adrenal gland did not fail in its action. The pituitary of the burned dog was destroyed by haemorrhage (as demonstrated macroscopically and histologically) and so did not respond to the stimulus of potassium chloride by increased output of ACTH. Increased output of ACTH was found in the normal dog. So the fault is lying at a supraadrenal level and in the pituitary gland itself. In none of the other experiments adrenocortical failure due to burn was found.

Another observation was that ACTH injected into the adrenal artery had a greater stimulating action in the burned dog than in the normal one and 40 units of ACTH could increase the corticoid content of the adrenal venous effluent further over the already increased corticoid level due to the burn trauma itself. Such feature was not only found after ACTH injection but also was observed after injecting different potassium salts in burned dogs. Potassium acetate and bicarbonate were better adrenocortical stimulants than potassium chloride. Potassium nitrate had a stimulating action inferior to potassium acetate and bicarbonate.

In the same investigation, high levels of bio and chemocorticoid content of the adrenal venous effluent were found in burned dogs. Hume *et al.* (1956) found in creased level of 17-OHCS in plasma and urinary excretion of the same was high during the first two weeks in the more extensively burned patients.

During the first few days after burning, the urinary excretion of 17-ketosteroid is greater than that found afterwards and it is maximum during the second or third day (Cope *et al.*, 1943; Wardlaw, 1955). Hardy (1955) mentioned that in relatively mild burn, a normal level of 17-ketosteroid excretion is found.



Increased excretion of corticosteroids is commonly found in burned subjects and it is more so in extensively burned patients (Heard *et al.*, 1946; Tompsett and Oastler, 1947; Talbot *et al.*, 1947; Evans and Butterfield, 1951; Browne, 1951; Roy, 1953; Hardy, 1955; Hume *et al.*, 1956).

#### Anaesthesia and operative trauma :—

17-Hydroxycorticosteroid in the blood rises in some cases (though the magnitude of rise is small) even before anaesthesia and operation and this is due to patient's psychological tension or fear for operation. This has been also found by Franksson *et al.* (1954). During anaesthesia and operation it rises further and stays at a higher level for 2 to 3 days after which it comes down. The blood steroidal level comes down to normal within a short period after operation and the following may be the reasons for it :—

- (a) Short lasting stress after operation.
- (b) Exhaustion either at the adrenal or at the hypothalamohypophysial level or at the controlling centers for the hypothalamus; but that this is not so, at least in the majority of cases, is proved by good adrenocortical response after ACTH injection and also after a second operation (where required) when blood 17-Hydroxycorticosteroid level rose again.
- (c) The formation and secretion of ACTH hormone from the pituitary may be at fault and high level of 17-Hydroxycorticosteroid in blood may lower the ACTH secretion.

Acute adrenocortical insufficiency occurs in some postoperative patients and if substitution therapy is not started, these patients end fatally and here comes the importance of assessment of adrenocortical reserve of patients pre-operatively.

After burns, compound fractures and intramedullary pinning operations, the blood 17-Hydroxycorticosteroid level remains high for many days. The reason may be that the stress remains for a longer period.

Patients having crushed injuries of extremities are sent to hospitals with tourniquets applied around the injured limbs. These patients are in extremely shocked condition. The blood 17-Hydroxycorticosteroid level is low in them. It rises to a very high level when the tourniquet is removed, the wound is attended and the treatment for shock is done. The subsequent rise after the initial low level of the blood corticoid may be due to the release of tissue breakdown products into the general circulation after the removal of the tourniquet. The neurogenic impulses from the site of injury and anxiety and fear in these patients are not stimulating the anterior pituitary sufficiently to discharge more ACTH. Franksson and Gemzell (1953) and Franksson *et al.* (1954) found low 17-Hydroxycorticosteroid level in the blood after traumatic shock. They suggest that the immediate circulatory disturbances in the pituitary gland inhibit the production or secretion of ACTH. The adrenal glands in patients dying very soon after accidents show minor alteration and the cortical lipoid store is good and slight depletion is very rarely noted. On exa-



mination of such a section of the adrenal gland, it is understood that the patient had no time to utilize his own adrenal steroid.

Roy (1953) found that burned subjects died with very high levels of urinary corticosteroids and in children the levels are very high. In lesser degree of burns, the urinary corticosteroids were proportionately high but not up to the standard that is found in moderately or severely burned patients in whom no difference in the high adrenocortical response could be made. Patients surviving after burns showed gradual lowering of the urinary corticosteroid value afterwards. During complications or operation the value is high again. Hardy (1955) also considered this point and mentioned "both moderate and severe burns respond in a similar fashion in regard to the excretion of corticosteroids."

#### **Eosinophil count in burn :—**

Evans and Butterfield (1951), Wight *et al.* (1953) and Hardy (1955) found depression of eosinophil count after burns. Sevitt (1951, 1954) found the eosinopenia to last only for one or two days in patients with burns of less than 10% of the body area and in more severely burned patients the eosinopenia lasted for 3 to 5 days. The eosinophil count was at a low level for weeks in very ill patients but the level was higher than that found in the immediate post-burn period.

Subsequently eosinopenia could be found during operations, change of dressings, infections or other stress factors. My personal observation is that in lesser degree of burns, there is a close correlation of the burn with the fall in the eosinophil count and the duration of eosinopenia is short. The fall in the eosinophil count is complete or nearly complete in severe burn and the duration of eosinopenia is prolonged indicating prolonged adrenocortical hyperactivity. Eosinopenia was found during change of dressings, in wound infection, during and after skin-grafting, after thrombophlebitis and lung-complications and during other sorts of complications. This shows that adrenals in burn are well responsive to subsequent stress. Burn cases showing prolonged eosinopenia without rebound phenomenon are prognostically bad.

#### **Burn and neurosecretion :—**

Theobald and Verney (1935) said that antidiuretic hormone is released after different stimuli and specially after pain. Baar (1956) isolated a peptide from the urine of burned patients and this resembled antidiuretin but it showed poor antidiuretic activity in rats. Hellmann and Weiner (1953), Itoh and Kimura (1953) and Itoh (1954) found the antidiuretic potency of urine, sweat and serum to increase after heat exposure. Itoh (1954) reported depletion of antidiuretic activity in the posterior pituitary glands of heat exposed rats in winter but in summer it was not so, though there was a high level of antidiuretic substance in serum in both the seasons. Gersh (1939) observed hypertrophy of parenchymatous cells in the neural lobes of heat exposed rats. Ortmann (1951) found increased reduction of Gomori substance due to dehydration in a hot environment. Ueno (1957) studied histological changes in the Gomori substance in the posterior pituitary lobes and the supraoptic and paraventricular nuclei in heat or



cold exposed rats. Gomori substance was extremely reduced in the hypothalamic cells on heat exposure and specially in those rats whose body temperature had been much elevated. ADH production and release are both increased in hot environment. Seasonal variation in the discharge of Gomori substance from the neurohypophysis of heat exposed rats could however be detected by Ueno (1957). Exposure to heat gave rise to severe changes in the pituicytes and interstitial tissues. It was also inferred that a great increase in ADH discharge occurred and this protected the rise in body temperature on heat-exposure.

The number of vacuolated cells is increased in burned animals (dogs, guineapigs). The vacuoles are usually spherical but they are also found to be compressed. Jewell (1953) noticed that the vesiculated cells were as heavily loaded with secretory granules as the normally looking cells ; but I have found that the secretory granules (CAHP stain) are definitely less in the vacuolated neurons. In the axons the CAHP positive material is found. The vacuoles are intracellular but surrounding a cell such a vacuolated appearance is sometimes found. The vacuoles contain neither fat nor CAHP positive material. At one end is a neuron filled up with neurosecretory material and at the other end is one with depletion of neurosecretory material and a vacuole. Thus the whole chain of events is due to the production and supply of the neurosecretion.

The hypothalamus, neuro and adenohypophysis are congested after burns.

The neurosecretory material is depleted from the neurohypophysis after burns and it is found in the blood vessels of neurohypophysis. The neurosecretory material is found also in the primary capillary plexus and in the anterior pituitary.

Examination of the hypothalamus and pituitary at different days in the post-burn period shows different stages of activities in these depending on the hypothalamoneurohypophysial and hypothalamoadenohypophysial activity. It has never been found that all the supraoptic and the paraventricular nuclei are in a vacuolated stage. So failure of the hypothalamus cannot be mentioned from these observations.

#### Morphological changes in the anterior pituitary after burns :—

The weight of the pituitary is diminished in severely burned patients and it is hyperaemic (Roy, 1953).

Selye (1950) found that after more chronic treatment with different types of alarming stimuli, the number of basophils was increased and the cells have a "signet ring" appearance.

In rats subjected to stress, Finerty *et al.* (1952) could not find alteration either in the percentage of hypophysial cell types or in the number or appearance of beta or delta types of basophils. After stress they found increase in methylene blue-staining cytoplasmic basophilia in the acidophil cells. There was a marked increase in the acid hematin stainable cytoplasmic granules in the acidophil cells, 3, 12 and 24 hours after scalding.



Roy (1953) found that in burned subjects and dogs, the number of basophils is increased. The following histological changes were also noted in the anterior pituitary after burns :—

- (a) Haemorrhage.
- (b) Blood spaces are full of blood.
- (c) Cellular disintegration with formation of lumina.
- (d) Excessive amount of colloid.
- (e) Vacuolar change in the cytoplasm of basophil cells.

The histological picture denotes an increased activity in the basophil cells, and since ACTH is localized in the basophil cells (Marshall, 1951) it may be inferred that increased ACTH secretion took place and there was no evidence of failure except where the pituitary is grossly damaged by haemorrhage. Increased adrenocortical activity (depletion of cortical lipid) was noted.

Degranulation of the periodic-acid-Schiff positive mucoid cells which are mainly basophils is an index of secretory activity (Pearse, 1952 ; Currie and Symington, 1955a, 1955b ; Symington *et al.*, 1955). Symington *et al.* (1955) found that the relative number of degranulated cells was increased in severely burned subjects and in those who were subjected to recent stress.

Shanklin (1956), however, found a reduction in the number of basophil cells in the human and dog pituitary after burns and also noted degranulation of cells by PAS technique. There was prominence of the acidophil granules in the central part of the gland and at the periphery many acidophil and chromophobe cells were in different stages of degeneration.

#### **Morphological changes in the adrenals in burned subjects :—**

Changes in the adrenals of burned persons have been described by Roy (1953). The adrenals are generally congested and hypertrophied. Haemorrhage destroying only a small part of the adrenal has been noted ; but severe haemorrhage with total loss of adrenal tissues has been rarely found though this has been described by Harris (1929) and Sevitt (1955) found it in 2 out of 82 autopsies.

#### **The types of lipoid loss :—**

Death occurring within a few hours after burn—In this group, practically there is no loss in the sudanophilic substance from the human adrenal glands.

Loss of sudanophilic substance, either moderate or severe, occurs when death takes place after 24 hours. The same has been observed by Sevitt (1955). Inversion of lipoid pattern is found few days or weeks after burns.

#### **Cellular disintegration and lumina formations :—**

These changes in the cortical parenchyma have been described in detail (Roy, 1953). These were found rather in patients dying 24 hours after burn than in those who died earlier. Roy (1958) has stated that such types of histological findings indicate increased adrenocortical activity.



Rich (1944) described these in diphtheria and in meningococcal, streptococcal and pneumococcal infections and postulated a relationship between this type of degeneration and the circulatory collapse found in some of the patients. Sevitt (1955) could not confirm Rich's postulate and he found that these histological findings are generally associated with adrenocortical hyperactivity and thus this change cannot be accepted as evidence of physiological failure of adrenal cortex. Roy (1953) found increased urinary corticosteroid excretion in patients dying after burns and the adrenal venous corticosteroid content was high in burned dogs. In these burned human subjects and dogs, the adrenal cortex showed cellular disintegration and lumina formations. So from these it may be inferred that such histological findings express increased adrenocortical activity and not a failure.

The cellular disintegration was not only found in the fascicular zone but also in the glomerular zone (Roy, 1953). This indicates increased aldosterone secretion from the glomerular zone.



## PATHWAYS BY WHICH THE STRESS MESSAGES TRAVEL AFTER BURN TO GIVE RISE TO INCREASED SECRETION OF ACTH AND ADRENAL HORMONES

Different types of mechanism are there which increase the adrenocortical activity in stress. In burn, possibly, a combination of the different types of mechanism is required for the increased secretion of ACTH and adrenal hormones. The mechanisms are mentioned below :—

(a) Sayers' peripheral humoral concept. Increased utilization of cortical hormones at the site of burn leads to increased discharge of ACTH which in turn stimulates the adrenal cortex to secrete more cortical hormones.

(b) Long's adrenaline hypothesis. In response to burn injury, increased secretion of adrenaline occurs, which then, stimulates the adenohypophysis to secrete more ACTH.

(c) Hypothalamic hormone(s) and histamine carried by the pituitary portal vessels to the adenohypophysis stimulate it to secrete more ACTH.

(d) Well co-ordinated action in between anterior and posterior hypothalamic nuclei and tuberal nuclei in adenohypophysial ACTH discharge.

(e) Afferent nerve impulses from the burned area reach via collateral connection through the reticular formation to the posterior hypothalamus. The adenohypophysis is activated from the posterior hypothalamus.

(f) There may be a release of ACTH which is present in the infundibular process.

(g) The hypothalamus is controlled by the frontal lobe of the brain, rhinencephalon, basal ganglia and mesencephalon.

(h) Chemicals liberated at the burned area are transported by the systemic circulation and these may stimulate the adenohypophysis to secrete more ACTH.

(i) Potassaemic hypercorticism may exist in burns (Roy, 1953), which also explains increased aldosterone secretion in this condition.

(j) Release of neurosecretory substance from the infundibular process and its entry into the adenohypophysis through vascular connections between the two may occur. The neurosecretory substance then stimulates the adenohypophysis to discharge more ACTH. Presence of neurosecretory material in the adenohypophysis and in the junctional area in between the adenohypophysis and the infundibular process has been observed by me in burns.

(k) Efferent stimuli travelling down the spinal cord can stimulate the adrenal cortex.

(l) Influence of ACTH on the discharge of hypothalamic neurohormone(s).

(m) Vasoconstriction or vasodilatation of the adenohypophysis controlling the activity of the gland.

(n) There exists a central control of ACTH discharge by histamine. This has been explained before.



## CHAPTER IX

### CHANGES IN THE ADRENALS

The materials (adrenals) for this study have been taken from (a) human cases during postmortem examination (within 24 hours of death). This includes both males and females but excludes children. 15 cases dying immediately after the accident have been taken as controls. 45 males and 25 females dying after varying intervals of fractures and compound injuries have been studied to note the changes in the adrenals. Cases dying after burn and intestinal obstruction have also been examined.

(b) Guinea pigs are of both sexes and 300-450 gm. in weight. They received ascorbic acid (5 mg. per 100 gm. of body weight) by subcutaneous injection once a day for 5 days before the experiment. They have been taken 8-10 in each group of experiment for short-term experiments. The adrenals have been taken out and kept in the proper fixatives for use afterwards.

(c) Male dogs are of 10-15 kg. in weight.

The study of the adrenals in the guinea pigs includes :—

- |  |   |              |
|--|---|--------------|
| 1. Birefringence                               | } | Rt. adrenal. |
| 2. Sudan IV stain                              |   |              |
| 3. Schultz reaction                            |   |              |
| 4. Histological demonstration of ascorbic acid |   |              |
| 5. Haematoxylin and eosin stain                | } | Lt. adrenal. |
| 6. PAS reaction                                |   |              |
| 7. Ribonucleic acid                            |   |              |

The study of human adrenals includes 1, 2, 5, 7 of the above. The fixatives used are formalin and susa depending upon the type of study. The study of the dog adrenals includes 2, 4, 5.

#### Staining Methods :—

For R.N.A.—Brachet's method (from Pearse, 1954).

For PAS technique—Hotchkiss (from Pearse, 1954).

For Ascorbic Acid—Bacchus (1950) (from Pearse, 1954).

The method of study in the animals is as follows :—

The stresses include femur fracture, burn and intestinal obstruction. These experiments have not only been carried out on normal animals but also on neurectomized and pituitary stalk sectioned dogs. Guinea pigs have not been used for long-term experiments. After these operations sufficient time is allowed for the adrenals to come to the normal stage after which the stresses have been applied and then the adrenals are examined.

#### Types of lipid depletion :—

(a) Patchy loss of lipids :—This means that areas of lipid loss alternate with areas of lipid filled cells.



(b) Near-total loss of lipids from the adrenal zones.

(c) Inversion of lipid pattern :—In this type there is loss of lipids from the outer zones but the lipids are present in the inner zones. This change has been described by Sarason (1943), Sevi (1955) and Symington *et al.* (1955). Symington *et al.* (1955) said that, "The significance of lipid reversion is not understood, but its similarity with the pattern found after large doses of cortisone therapy suggests that this may represent, histologically, the process of so-called corticoid withdrawal and the change over from catabolism to anabolism (Moore, 1953)." This finding in the present work has been noted towards the end of the stress periods and so the possible explanation may be the same as has been offered by Symington *et al.* (1955). Loss of sudanophilic lipids from adrenals of rats after traumatic injuries has been found by Selye (1936a, 1936b, 1937), Oka (1939), Popjak (1944), and in man by Kalaja (1947).

Stolfi (1936), Long (1947), Berri (1948), Selye and Stone (1950) found loss of adrenal ascorbic acid concentration after trauma.

Enlargement of adrenal cortex and loss of sudanophilic lipids have been found in different animal species (Zwemer, 1936). Kabat and Hedin (1942) found hyperaemia and haemorrhages in the cat. There is rapid loss of adrenal cholesterol after burns in the rat (Harkins, 1944; Harkins and Long, 1945; Long, 1947; Ludewig and Chanutin, 1947; Sayers, 1949). In guinea pigs and rats, this is preceded by more rapid loss in ascorbic acid (Miyagi, 1939; Long, 1947; Ludewig and Chanutin, 1947; Sayers, 1949).

Stoner *et al.* (1953) has said, "A gland was, therefore, considered to be in the resting state if it contained abundant sudanophilic and ketogenic material in large droplets and if the birefringent crystals were numerous and large. A part of the cortex was considered to be actively secreting when there was loss of sudanophilic and ketogenic material and a reduction in size of the droplets and birefringent crystals. After ACTH administration, the reduction in the number and size of the birefringent crystals was particularly striking." My finding corroborates the view of Stoner *et al.* (1953) regarding the two different states of the adrenal gland.

The birefringence, Schultz reaction, and histological demonstration of ascorbic acid show the same type of change as with sudan stains (*i.e.* the loss and replenishment of birefringent crystals, cholesterol and ascorbic acid). The ascorbic acid has not been found in cells showing cytolysis, but in the starting of the process, the granules take up a peripheral arrangement surrounding the cytolytic area within the cell.

**Ribonucleic acid :—**This shows the same feature as ascorbic acid granules in the cells showing cytolysis; RNA granules are found to be increased in the inner cortical zones in animals with stress. The same feature is also found in human cases after compound fracture and burn.

**PAS reaction :—**The PAS positive substances in the form of granules in the cells may be found in the adrenals of normal guineapigs. But the granules are bigger and the cells containing the granules are more towards the end of the stress periods. Moreover, the plasma in the ad-



renal vessels is sometimes found to be intensely PAS positive. Selye and Stone (1950), and Selye (1950) thought about the occurrence of similar cytoplasmic inclusions as "expression of some derangement in the biology of the adrenocortical cell, possibly a disturbance occasioned by the induced disequilibrium between corticoids (and related steroids) on the one hand, and corticotrophic pituitary hormones on the other." (From Selye and Salgado, 1955.) Salgado and Selye (1954a, 1954b, 1954c) thought whether the accumulation of PAS positive cytoplasmic granules might be due to excessive mineralocorticoid secretion. Selye and Salgado (1955) described PAS—tingible bodies in adrenocortical cells of rats treated with various steroid hormones and discussed the functional implications of these morphological changes.

From the present work the functional significance of the PAS—tingible substances in the adrenocortical cells is very difficult to assess definitely but it may be an expression of the increased hormonal output and moreover when the glands change over to the resistant phase of the GAS.

**Haematoxylin and eosin stain :—**

The following changes have been encountered :—

- (a) Vacuolation and cytolysis.
- (b) Demonstration of basophilic granules in the cytoplasm of the cells of the zona fasciculata.
- (c) Congested appearance of the adrenals.
- (d) Formation of lumina and tubules within the cortex.

Zamcheck (1947), Selye and Stone (1950), Sevitt (1955), and Symington *et al.* (1955) found similar formation of lumina in the cortical parenchyma after injuries. The neurectomized dogs show less severe adrenal changes when subjected to different stresses and the depletion of sudanophilic substances and ascorbic acid granules is lesser than what is found in normal animals with different types of stress.

In pituitary stalk-sectioned dogs the sudanophilic substances and ascorbic acid granules are diminished in response to stress.

**Study of the human adrenals :—**

Birefringence, sudan stain and ribonucleic acid show the same type of change as has been described above. The haematoxylin and eosin stained preparations show the following :—

(1) **Death occurring within 24 to 48 hours :—**

- (a) Cortical hyperplasia.
- (b) Thickening of the capsule with regeneration of cells from the capsule.
- (c) Cytolysis found not only in the cortical parenchyma but also in the accessory cortex.
- (d) Lumina and tubule formation.
- (e) Congested appearance of the adrenals.

Regarding the lipid depletion, it is found that the accessory cortex is full of lipids, but the cortical parenchyma shows depletion of lipids. Such type of dissociation may be found which means that the activity is not same in the two situations.



(2) Death occurring after 48 hours to 10 days :—

- (a) Cortical hyperplasia.
- (b) Capsular thickening with cellular regeneration.
- (c) Fine fibrosis in the fascicular zone.
- (d) Cellular disintegration with vacuolar change, lumen formation and formation of tubules.
- (e) Round cell infiltration in the cortex.

**Conclusion :—**

The findings in this chapter corroborate those established with the help of biochemical estimations in the previous chapters. Moreover, different adrenocortical changes encountered after stress in guinea pigs, dogs and man have been stated.



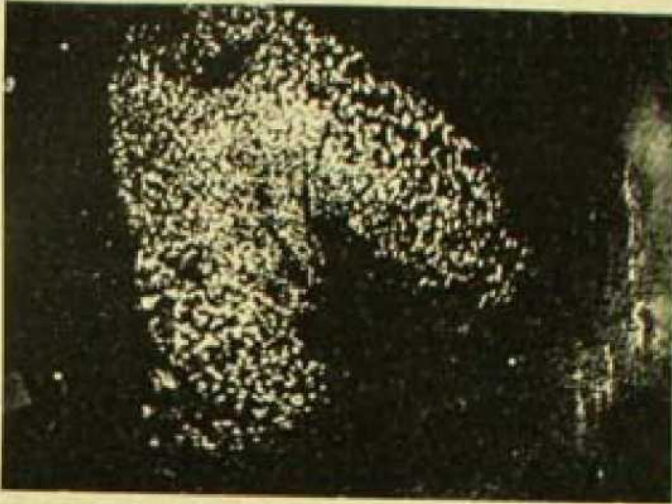


Fig. 23.—Normal guinea-pig's adrenal. The distribution of birefringent crystals is seen.



Fig. 25.—Guinea-pig's adrenal—multiple fracture at 3rd hour. It shows depletion of birefringent crystals. (Magnification  $\times 50$ ).



Fig. 26.—Guinea-pig's adrenal—neurectomy  $\times$  simple fracture at 3rd hour. (Magnification  $\times 50$ ).



Fig. 27.—Guinea-pig's adrenal—compound fracture 3rd day. (Magnification  $\times 50$ ).





Fig. 28.—Guinea-pig's adrenal—compound fracture 20th day. It shows replenishment of the birefringent crystals. (Magnification  $\times 59$ ).



Fig. 29.—Human adrenal from a simple fracture case. It shows patchy loss of birefringent crystals. (Magnification  $\times 50$ ).



Fig. 32.—Human adrenal from a burn case. Shows patchy loss of birefringent crystals. (Magnification  $\times 50$ ).





Fig. 33.—Human adrenal from a multiple fracture case. It shows patchy loss of birefringent crystals.  
( Magnification  $\times 50$  ).



Fig. 34.—Human adrenal from a burn case. Loss of birefringent crystals is seen.





Fig. 35.—Normal human adrenal.  
(Magnification  $\times 50$ ).

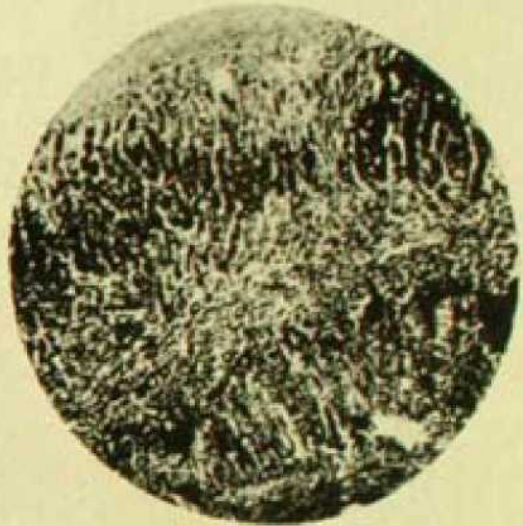


Fig. 36.—Human adrenal from a burn case.  
It shows patchy loss of sudanophilic  
substances.  
(Magnification  $\times 50$ ).

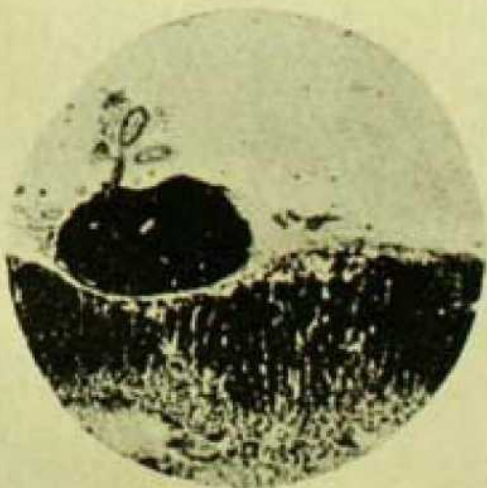


Fig. 37.—Sudan IV stain of the  
human adrenal gland and the acces-  
sory adrenal cortex. The accessory  
cortex does not show any deple-  
tion of sudanophilic substance but

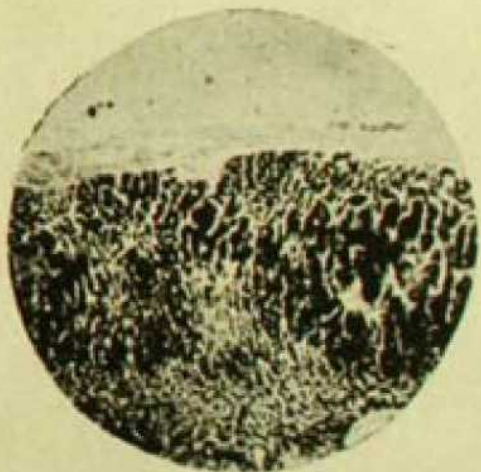


Fig. 38.—different portion of the  
same adrenal gland shows depletion.  
A case of simple fracture.  
(Magnification  $\times 50$ ).



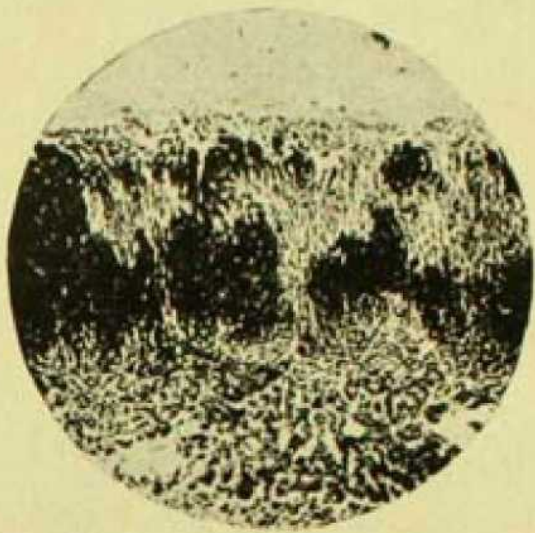


Fig. 39.—Human adrenal from a burn case showing inversion of lipid pattern i. e., loss of lipids from the outer cortex but presence of the same in the inner cortex.  
(Magnification  $\times 50$ ).

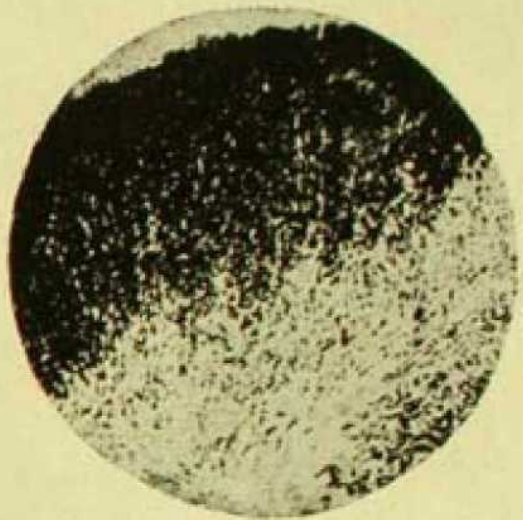


Fig. 41.—Normal guinea-pig's adrenal.  
(Magnification  $\times 50$ ).

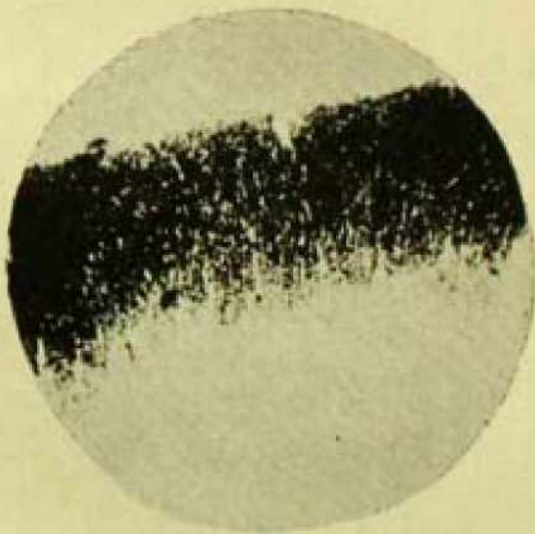
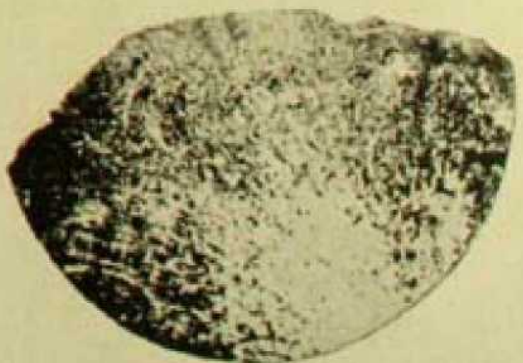


Fig. 42.—Guinea-pig's adrenal after simple fracture 3rd hour.  
(Magnification  $\times 50$ ).

Fig. 43.—Guinea-pig's adrenal. It shows loss of sudanophilic substance three hours after burn.  
(Magnification  $\times 50$ ).





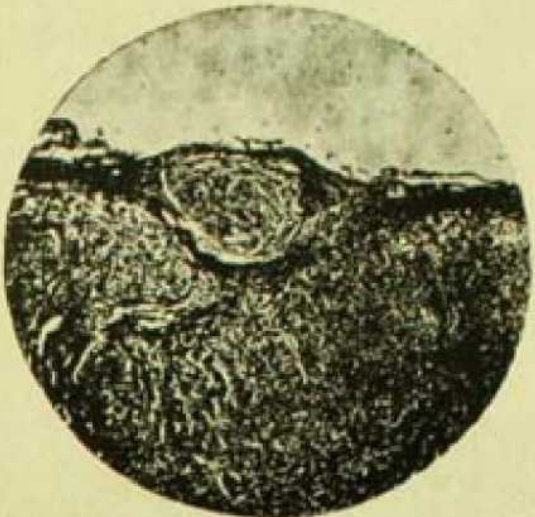


Fig. 44.—Human adrenal with accessory adrenal cortex.  
 (Haematoxylin and eosin stain  $\times 50$ ).

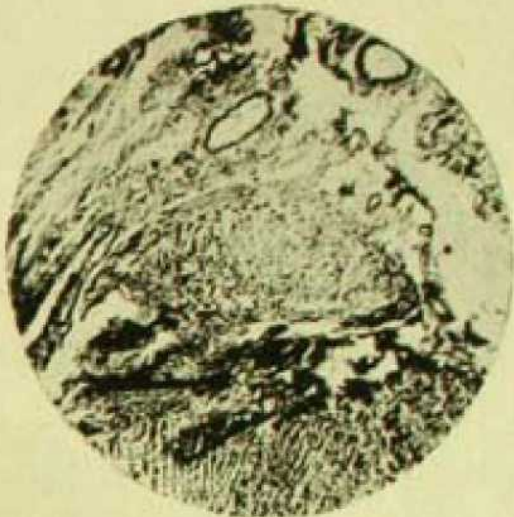


Fig. 45.—Human accessory adrenal cortex showing compact cells and cells with cytolytic changes (from a burn case).  
 (Haematoxylin and eosin stain  $\times 50$ ).

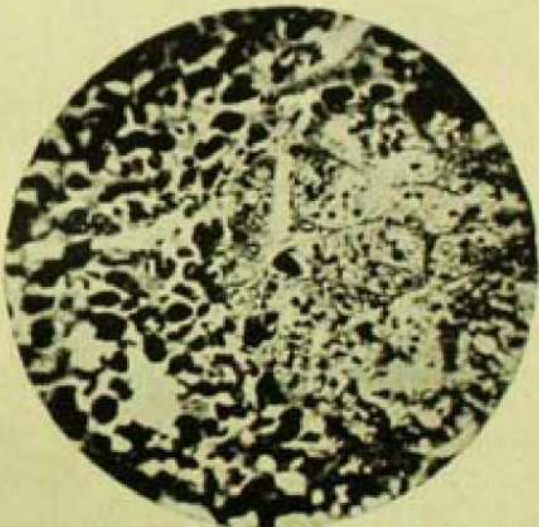
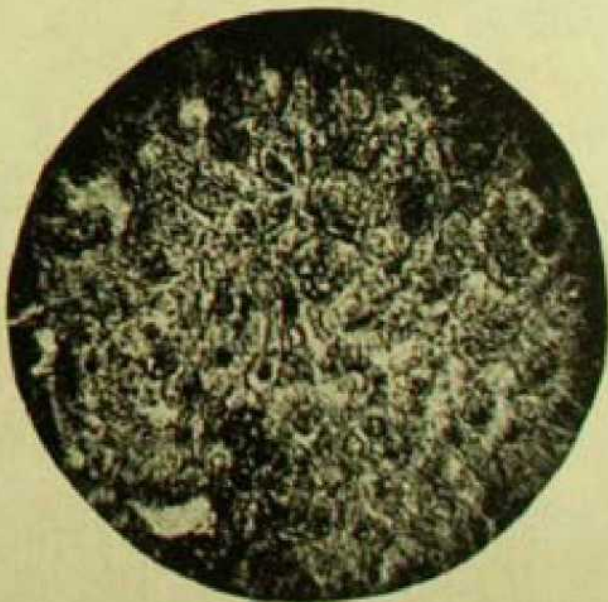


Fig. 46.—Accessory adrenal cortex of the figure 45 at a higher magnification showing the same features more clearly (Haematoxylin and eosin stain).

Fig. 47.—Adrenal of a normal guinea-pig.  
 (Magnification  $\times 450$ ).





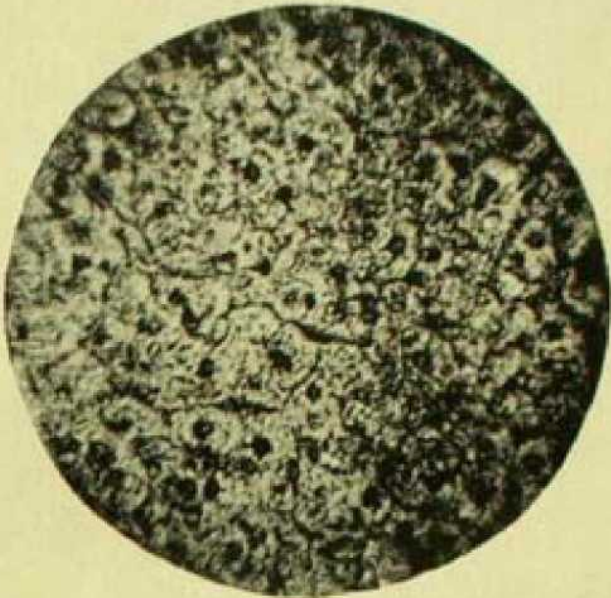


Fig. 48.—Guinea-pig's adrenal cortex 3 hours after simple fracture. It shows diminished A. A. granules and their peripheral arrangement in the cell.  
(Magnification  $\times 450$ ).

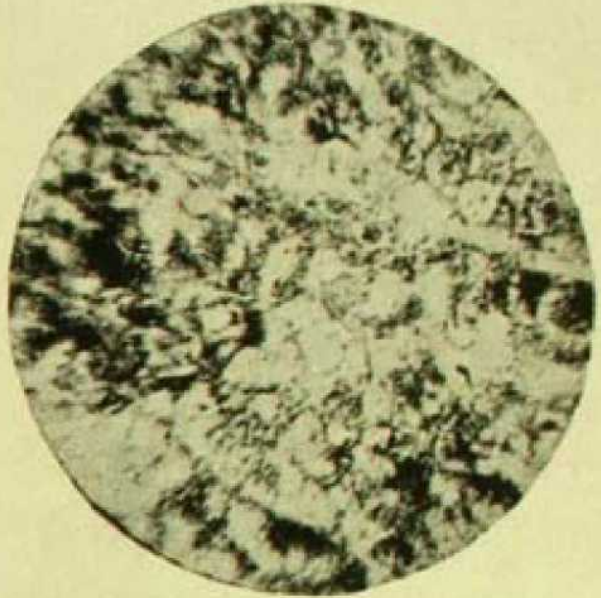


Fig. 49.—Guinea-pig's adrenal after intramedullary pinning (unilateral) 3rd day. A. A. granules are peripherally arranged in the cells; the cells also show vacuolar changes.  
(Magnification  $\times 450$ ).

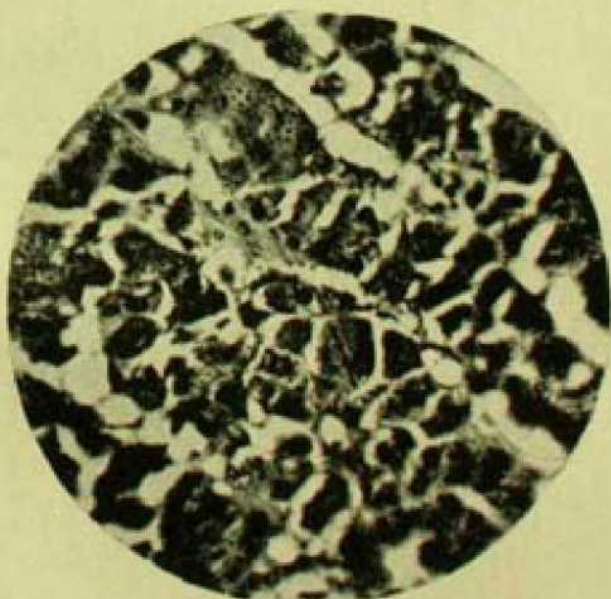


Fig. 50.—Basophilic granules in the adrenal cortical cells. (Guinea-pig's adrenal).  
(Magnification  $\times 450$ ).

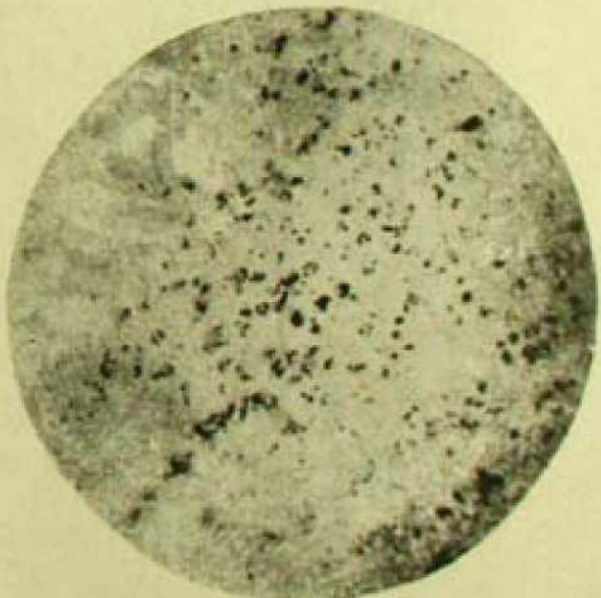


Fig. 51.—R. N. A. granules in the zona fasciculata and reticularis of human adrenal from a case of compound fracture.  
(Magnification  $\times 215$ ).



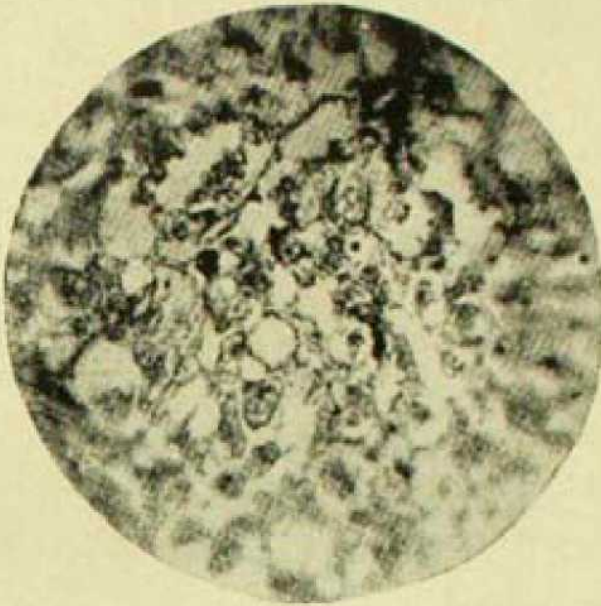


Fig. 52.—P.A.S. positive cells in the adrenal cortex of a normal guinea-pig. The cells are very few.  
(Magnification  $\times 450$ ).

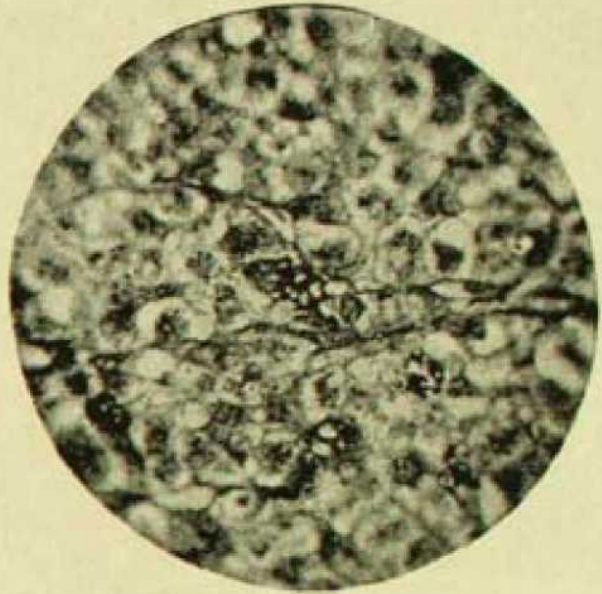


Fig. 53.—Increased P.A.S. positive cells in the adrenal cortex of a guinea-pig after compound fracture.  
(Magnification  $\times 450$ ).

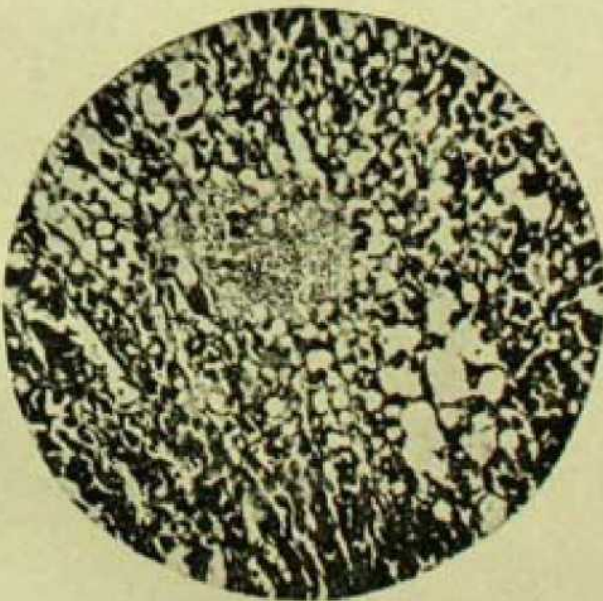


Fig. 54.—Human adrenal cortex showing cytolitic changes (lumen and tubule formation) from a case with intestinal obstruction.  
(H+E, stain  $\times 215$ ).

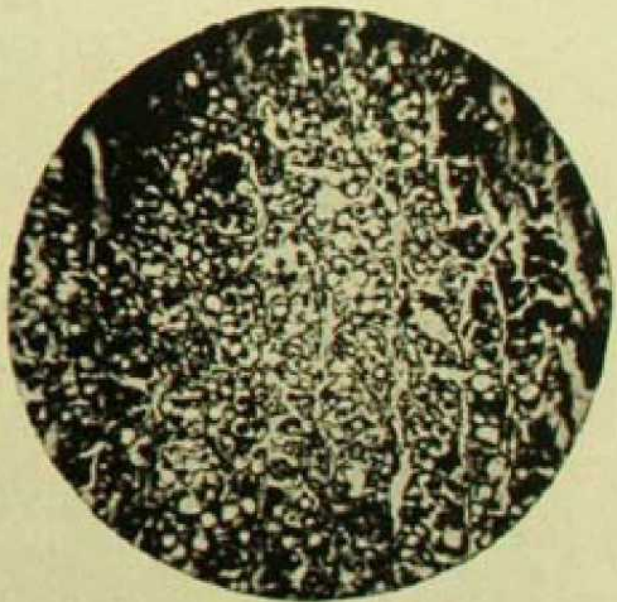


Fig. 55.—Cytolytic areas and normal areas in the same adrenal gland (human). From a case of multiple fracture.  
(H+E, stain  $\times 215$ ).



Fig. 56.—Cytolytic and normal areas in the same adrenal gland (human). From a case of multiple fracture.  
 (H+E. stain  $\times 215$ ).

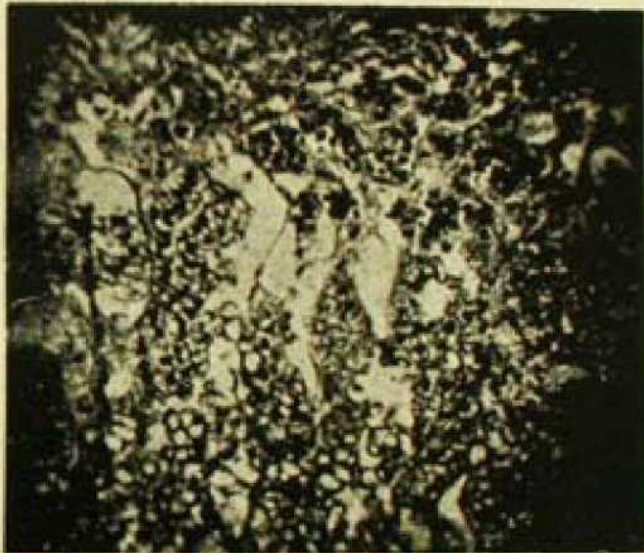
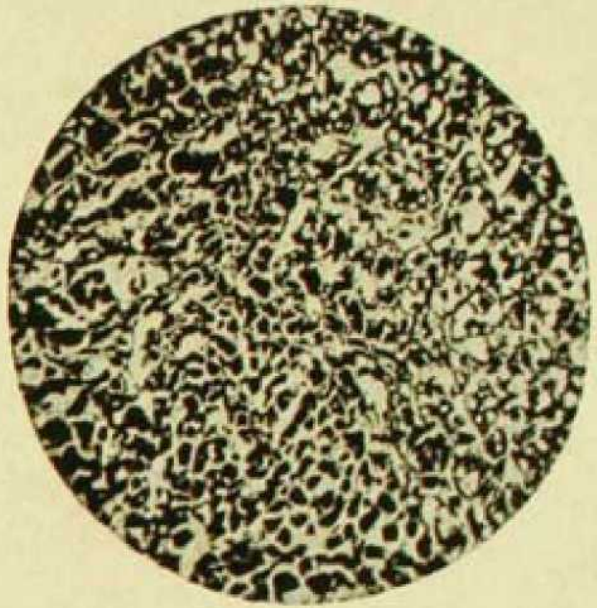
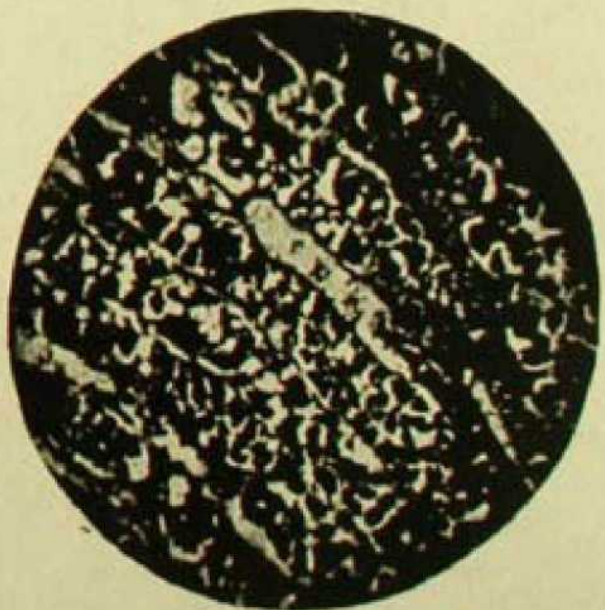


Fig. 57.—Tubule formation in the zona fasciculata (human). From a burn case.  
 (H+E. stain  $\times 215$ ).

Fig. 58.—Human adrenal from a case of multiple fracture showing tubule formation.  
 (H+E. stain  $\times 215$ ).





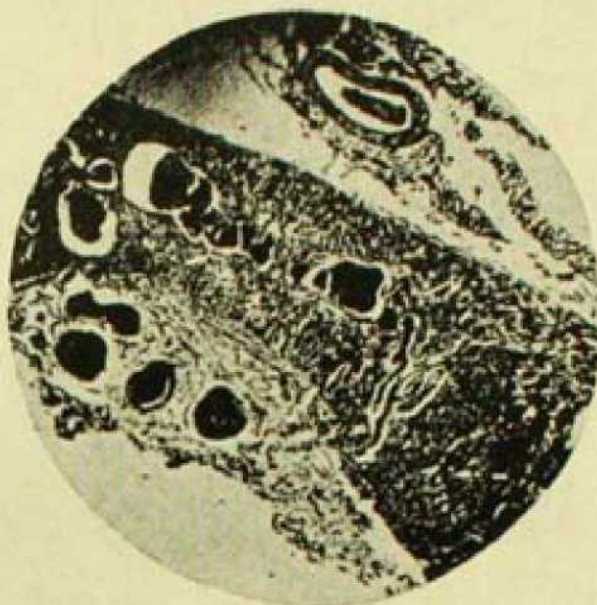


Fig. 59.—Congested appearance of the adrenal (human) from a case of compound fracture.  
 (H + E. stain  $\times 50$ ).

Fig. 60.—Round cell infiltration in the zona fasciculata of the human adrenal cortex. A case of compound fracture.  
 (Magnification  $\times 215$ ).

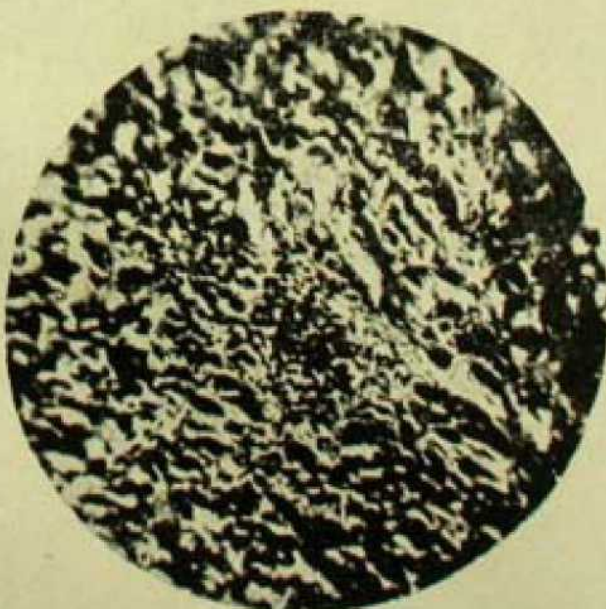
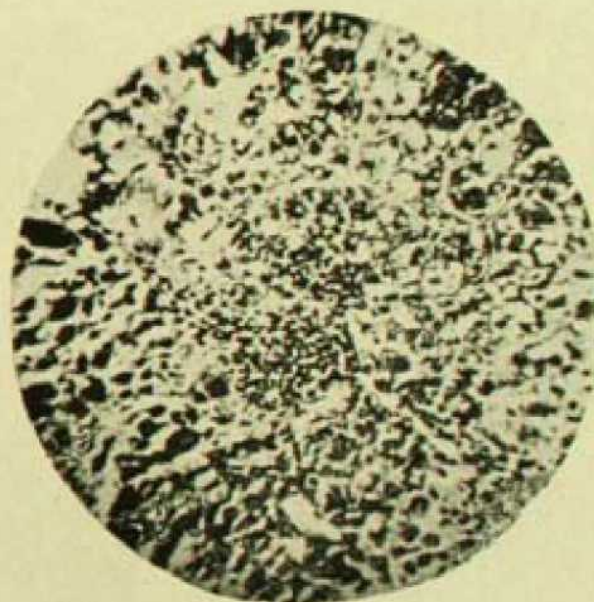


Fig. 61.—Round cell infiltration in the zona fasciculata of the adrenal cortex. Intestinal obstruction.  
 (Magnification  $\times 215$ ).



Fig. 62.—Guinea-pig's adrenal after simple fracture showing alveolar formation in the zona fasciculata.  
(H+E. stain  $\times 215$ ).

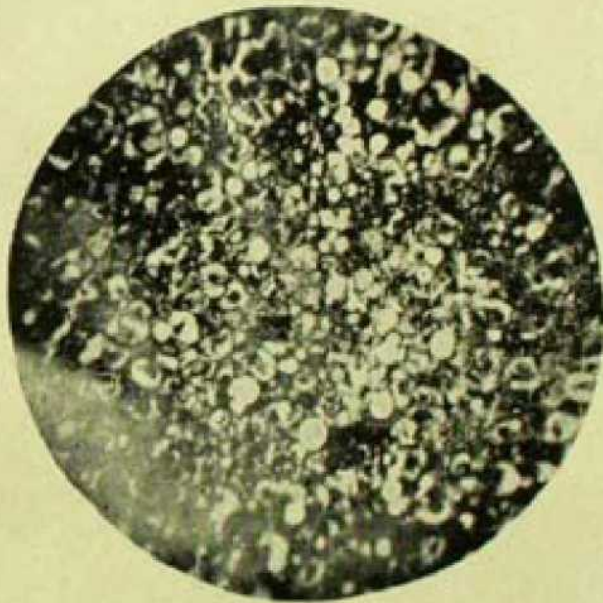
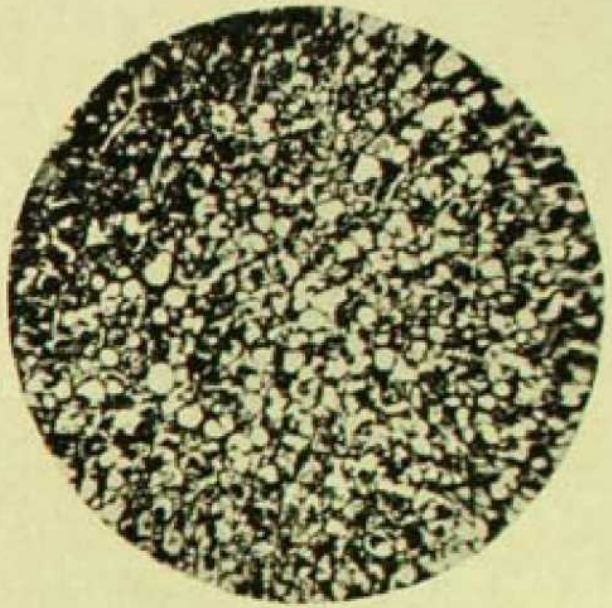
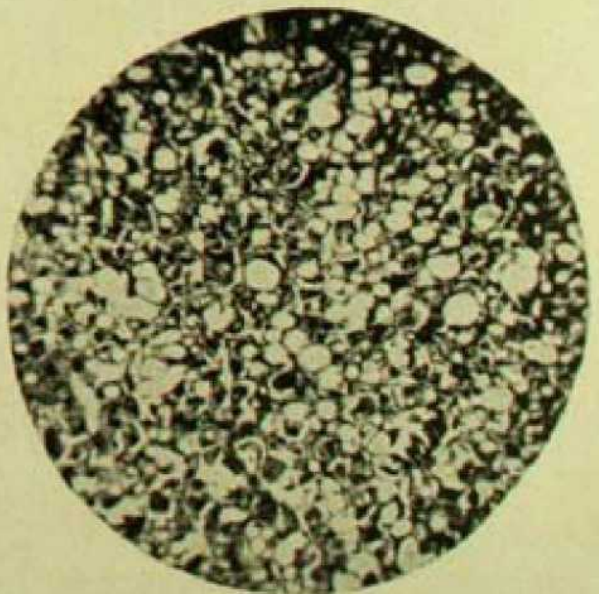


Fig. 63.—Guinea-pig's adrenal after multiple fracture showing cytolytic changes in the zona fasciculata.  
(H+E. stain  $\times 215$ ).

Fig. 64.—Guinea-pig's adrenal showing alveolar formation in the zona fasciculata after burn.  
(H+E. stain  $\times 215$ ).





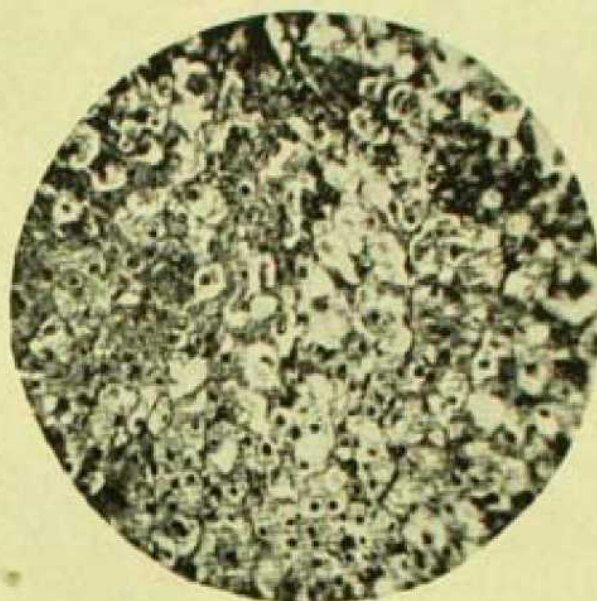


Fig. 65.—Guinea-pig's adrenal after unilateral (tibial) intramedullary pinning showing cytolitic changes in the fascicular zone.  
 (H + E. stain  $\times 215$ ).

Fig. 67.—Guinea-pig's adrenal after bilateral intramedullary pinning (tibial) showing alveolar formation and round cell infiltration in the zona fasciculata.  
 (H + E. stain  $\times 215$ ).

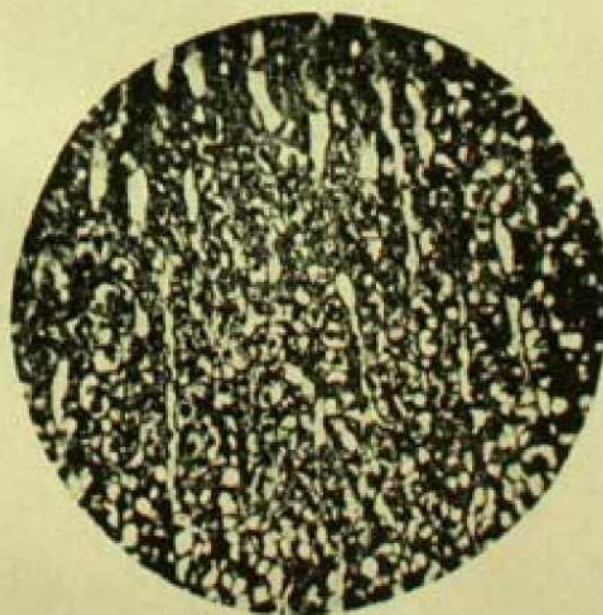
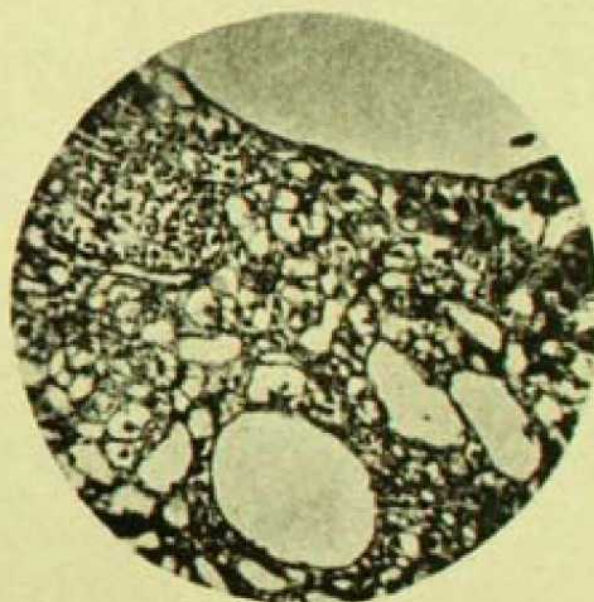


Fig. 68.—Dog's adrenal after multiple fracture. It shows tubule formation in the fascicular zone.  
 (H + E. stain  $\times 215$ ).



## CHAPTER X

### FINAL CONCLUSION

The different types of orthopaedic cases have been taken up to study the adrenocortical activity.

The experimental set-up has been so adjusted as to study some of the pathways by which the adrenal is brought into increased activation after stress. The two components of the pathways—nervous and vascular have been considered. When both the components are present, stresses give rise to good adrenocortical response. After different types of stress in a deafferented (both sympathetic and somatic) limb, the response can still be elicited. In dogs with closed loop intestinal obstruction and denervation of the loop, there is increased 17-hydroxycorticosteroid output. This proves that in the absence of the nervous pathways, the vascular pathways are competent to carry the stress message. In the animals in which both these pathways have been blocked, response after fracture has not been elicited.

In midbrain sectioned dogs, the rise in the 17-hydroxycorticosteroid output after stress is very small.

Pituitary stalk section has been done in dogs. These animals when subjected to stress show increased adrenocortical activity; but purely psychic stress requires the presence of the hypophysiportal vessels for the response.

Regarding the humoral substances responsible for the increased adrenocortical activity, the role of histamine has been investigated. Other chemicals liberated as a response to trauma may equally act; but these have not been investigated in the present work. Histamine carried by the systemic circulation and the hypophysiportal vessels is a good stimulant for the adeno-hypophysial ACTH discharge. Central control of ACTH discharge by histamine has been described.

Question of adrenocortical failure in stress has been discussed.

The histologically demonstrable neurosecretory substance has been studied in different experimental preparations of the animals after stress.

From the above it is evident that the avenues by which the stress message can elicit the increased adrenocortical activity is diverse and consideration of a single factor is not correct.

Histological study of the adrenals after stress has been done and the changes have been mentioned.



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## PART II



## CHAPTER I

### SECTION A

# BRAIN MECHANISMS RESPONSIBLE FOR ACTH RELEASE IN EXPERIMENTAL BURNS (1966)

The cerebral cortex of the dog possesses an inhibitory influence over the pituitary-adrenal-axis. Rise in adrenal venous 17-OHCS output occurs after decortication operation. The output further rises after burn trauma. After brain removal up to the level of thalamus there is increased adrenocortical response after burn. In solitary pituitary experiments with supratentorial brain matters removed, increased adrenocortical secretion has been noted. Adrenal venous 17-OHCS output rises further after burn trauma. The hind-brain-factor (HBF) is not very important for the increased adrenocortical secretion in solitary pituitary experiments as the same events have been noted after removal of the infratentorial structures. In this case there is increased adrenocortical activity and 17-OHCS output further increases after burn trauma.

### INTRODUCTION

In the second half of the nineteenth century Claude Bernard said that one of the most important features of all living beings is their ability to maintain the constancy of their internal *milieu* in spite of changes in the environment. The different defense reactions to maintain a stable internal environment has been called by Cannon as 'homeostasis.' This is achieved through the excess liberation of adrenalines. In 1915 he mentioned, 'It is a matter of prime importance for further discussion to determine whether the adrenal glands are in fact roused to special activity in times of stress.' He found the evidence that adrenal secretion (adrenin) was increased in emotional excitement and painful stimulation. Selye (1936) attached importance to the pituitary-adrenocortical system. The totality of the damage and the body's adaptive reactions has been called by Selye as stress syndrome or general adaptation syndrome. It has got three stages : (1) the alarm reaction ; (2) the stage of resistance ; and (3) the stage of exhaustion. The neuroendocrine system is important in maintaining resistance during stress.

The following theories have been put forward to explain the mechanism of adenohipophyseal ACTH release :

(1) Feed-back mechanism of Sayers (Sayers and Sayers 1947, 1948 ; Sayers 1950). The circulating level of adrenocortical steroids in blood controls ACTH secretion.

(2) Reflex discharge of adrenaline from the adrenal medulla controls ACTH release (Long 1947, 1952 ; Gershberg *et al.*, 1950 ; McDermott *et al.*, 1950).



(3) ACTH release is controlled by hypothalamic influences through hypophyseal-portal vessels (de Groot and Harris 1950; Harris 1950; Harris and Jacobsohn 1950; Harris and Fortier 1954; and others).

Karplus and Kreidl (1909, 1910, 1912) first described that the hypothalamus had some bodily functions. On stimulation of the walls of the third ventricle there was acceleration of the heart-beat, changes in the movements and secretion of the gut, dilatation of the pupils and other autonomic effects. Cerebral hemispheres were removed from many of their experimental animals for destroying the projection fibres. On stimulation of the wall of the third ventricle in such animals the same reactions were found. Local anaesthesia applied to the part to be stimulated abolished the reactions. They also traced the paths which were essential for the mediation of the response. Cardiac responses depended upon the ventral roots of the upper five or six thoracic segments. Stellate ganglionectomy blocked the response.

Houssay and Molinelli (1925) stated that epinephrin secretion by the adrenal medulla is controlled by the hypothalamus.

Hess (1925, 1932, 1938, 1948, 1954) stressed upon the importance of the hypothalamus and he developed a method for stimulating different parts of the diencephalon in conscious cats by indwelling electrodes. After the stimulation experiments were over, the particular area of the brain was lesioned and the effects were observed.

Bard (1928) stated that the hypothalamus was important for rage reactions in cats. 'Sham rage' in a decorticate cat is not present when the connection between the hypothalamus and the brain stem is disrupted. Thus in the caudal hypothalamus of the cat there is a centre which controls the emotional mechanisms, *e.g.* the angry behaviour.

Cannon's (1932) work on emergency reactions and homeostasis laid importance on the sympathetic nervous system.

The hypothalamus is not only an important nodal area which is related to behavioural and emotional responses but also it is important as a centre which regulates the endocrinological activities of the body.

Selye (1954) says that the different endocrine glands are important for the elicitation of the General Adaptation-Syndrome. The adaptation also occurs at a higher level through the mediation of the different areas of the central nervous system. Lissák and Endrőczy (1960) stresses the importance of the central nervous system in the adaptation activity. Elkes (1962) thinks that the septal region, the median eminence of the hypothalamus, the periaqueductal grey and other unidentified regions act as important areas for maintaining equilibrium. Upward influences come from the brain stem and downward influences project from the cortex and the caudate and lentiform nuclei. The various pathways that are required for appropriate homeostasis are as yet not very clear. These responses include endocrine, somatomotor and visceromotor effect. The role of hippocampus and amygdala in the activity of the pituitary-adrenal-axis is in the process of recent evaluation. This mechanism is important for adaptation activity after stress. Elkes further states, 'Indeed, stress response may be manifest not only as overactivity (resulting from the



faltering of a physiological braking mechanism) but also as underactivity (resulting from excessive inhibitory tone fired, in turn, by a runaway excitatory process). It is noteworthy that in the underactivity and withdrawal of extreme depression, the levels of hydroxycorticoid are apt to be high rather than low. Stress response may thus travel in different guises; and it will be well not to take overactivity and underactivity at their face value in judging such a response.'

Richter (1958) thought that Selye's (1950) 'stress concept' did not attach any importance to the nervous system in response to stress. However, in 1954 Selye explained this position. Richter also thought of the possibility that the nervous system might be affected by stress. Very severe stress leads to changes in the hypothalamic nuclei and these lesions explain for the different types and special combinations of the responses to stress. Also these may produce mental and physical diseases with periodic manifestations. For maintaining homeostasis the hypothalamic cells were markedly stimulated. Ultimately there was exhaustion of the hypothalamus with production of lesions. Some swim-stressed rats actually developed pituitary tumours.

Roussy and Mosinger (1946) in their *Traite de Neuro-Endocrinologie* said that they were forced to demonstrate the existence of a reflex hypophyseal neuroregulation as a result of concentrating their study on the sum total afferent nervous paths to the excitosecretory centres of the hypophysis. On page 333 they mention that the cingulate gyrus has to influence the functional role of both the hypophysis and epiphysis.

The adenohypophysis does not get any direct nervous conrection of any functional significance with the hypothalamus (Okada *et al.*, 1955; Palay 1957; Farquhar and Rinehart 1957; Harris 1958). However, direct functional neural connection to the adenohypophysis has been thought to exist by Vazquez-Lopez and Williams (1952), Metuzals (1956), Smith (1956), and others.

At present extrahypothalamic structures controlling the activity of the pituitary-adrenal-axis are in the process of exploration (Endröczy and Lissák 1960; Endröczy *et al.*, 1954; Martin *et al.*, 1958, and others).

In this part the results have been presented regarding the influence of hypothalamic and extrahypothalamic areas on pituitary-adrenal-axis after burn (ablation studies). This also includes the solitary pituitary experiments.

#### BRAIN LESIONS AND ACTH SECRETION

Hume and Egdahl (1959) summarized the reports of Egdahl *et al.* (1958) and Egdahl *et al.* (1959) where it was shown that even after removal of the brain the animals could respond to operative injury with rise in adrenocortical secretion. In one group only the cortex was removed and in the other group cortex and thalamus were ablated. Both these groups responded to operative trauma well. In a third group all the brain matters above the inferior colliculus were removed leaving behind the pituitary, cerebellum, pons and medulla. Operative trauma in this



group showed marked adrenocortical response on many occasions. In all the groups the resting levels of corticosteroid secretion was high. Hume and Egdahl (1959) concluded that the secretions of ACTH and adrenocorticosteroid after trauma were under the control of a complex neurohumoral mechanism situated in different areas of the brain.

Story *et al.* (1959) showed persistent elevation of corticosteroid secretion up to six hours after removal of large portion of the hypothalamus in some animals.

Egdahl (1960) studied adrenal venous 17-OHCS output and catecholamines in the basal condition and after burn trauma in dogs with isolated pituitary. Up to five days after operation these animals had high resting corticosteroid and two-thirds of the animals responded to the stimulus with increased 17-OHCS output. Low-resting catecholamine levels increased after burns. He postulated a HBF (hind brain factor) which passed through the systemic circulation and acted on the pituitary and thereby released ACTH. Possibility of a spinal cord neurosecretion is also there.

That the hind brain factor is not important is proved by the experiments of Wise *et al.* (1962) and those of Egdahl (1962) himself. He mentioned a period of depressed adrenocortical function after the preparation of isolated pituitary. Following this there was a persistent elevation of the basal secretion. Removal of total brain and spinal cord or removal of posterior pituitary or abdominal viscera did not change the response.

Egdahl (1961a) showed that the constriction of the inferior vena cava is a strong stimulus to the isolated pituitary.

Matsuda *et al.* (1963) removed the forebrain anterior to the superior colliculus in rats leaving behind the pituitary intact. Removal of the cerebral cortex and subjacent brain in the rat did not show any sustained rise in peripheral plasma corticosteroid. In the rat, according to the authors, cerebral cortical inhibition does not occur. For pituitary-adrenocortical activation in the rat at least the median eminence-stalk-pituitary complex should be present.

Matsuda *et al.* (1964) used animal preparations (rats) with removal of all forebrain anterior to the superior colliculus, but having median eminence, stalk and pituitary intact. In these animals adrenocortical response was increased after ether anaesthesia with or without trauma equal to that of normal controls. With nembutal anaesthesia trauma did not lead to a rise in adrenocortical secretion unless a large peninsula was left connecting the hypothalamus to the dorsal mesencephalon and remaining hind brain. Spinal cord section in otherwise intact animal under nembutal (but not ether) anaesthesia prevented the rise in adrenocortical secretion in response to a leg-break distal to the cord section. Ether directly stimulates the median eminence leading to increased ACTH release.

Wise *et al.* (1964) studied the effect of brain removal in dogs previously subjected to pituitary stalk section. The hypothalamus and other neural tissues above the midbrain were removed in 11 dogs and the effects on adrenocortical secretion were noted. They found that the response in



severely stressed dogs was not only due to CRF from the hypothalamus but it might be also due in part to an ACTH stimulating humoral agent liberated from traumatized tissues.

Endrőczy *et al.* (1963) observed an increase of ACTH secretion in cats after cholinergic chemical stimulation of the medial and caudal hypothalamus as well as the posterior hypothalamus and ventral tegmentum. No significant change in ACTH secretion occurred after stimulation of the hypothalamo-pituitary-neurosecretory system. This observation is at variance with the hypothesis that vasopressin or oxytocin plays an important part in the control of pituitary-adrenocortical activity.

Brodish (1963) said that in the rat the entire area of the ventral hypothalamus extending from the optic chiasma to the mammillary bodies is involved in ACTH secretion. A diffuse hypothalamic nucleus or network controls ACTH secretion. The centre is not a localized and discrete one.

Hume and Jackson (1959) observed that for a period of time varying from four hours to seven days after hypothalamic destruction the adrenocortical response to trauma was intact. From seven to 14 days there was loss of normal response to trauma but ACTH response was there. Spinal cord section abolished the response to trauma in the normal dog.

Witt and Keller (1960) performed hypothalamectomy and midbrainectomy on dogs. These preparations survived total pancreatectomy performed two to four weeks after brain lesions and without any hormonal supportive therapy. There was diabetes mellitus. Adrenal cortices were not atrophied, rather in some animals there was varying degree of hypertrophy.

Keller *et al.* (1954) found that ventral hypothalamectomy did not interfere with the eosinopenic response to surgery.

Stimulation of the thalamus or lower posterior hypothalamus (Suzuki *et al.* 1960) led to increased adrenocortical function. Stimulation of anteromedial paraventricular region of the hypothalamus had the same result (Mason 1958a). Stimulation of the thalamus (Mason 1958b) or putamen (Mason 1958a, 1958b) did not alter the adrenocortical function.

## MATERIALS AND METHODS

Male dogs of 10 to 15 kg in weight were used in this experiment. Intravenous nembutal anaesthesia was administered in a dose of 30 mgm/kg of body weight. Right lumbo-adrenal vein was cannulated after the method of Hume and Nelson (1955). Burn trauma was produced by immersing the extremity in boiling water (100°C) for 30 seconds under nembutal anaesthesia. Adrenal venous 17-OHCS has been estimated after the method of Silber and Porter (1954). Sterile techniques have been used all throughout the procedures except in the very acute experiments. Animals exhibiting intracranial infections were excluded from the series. In brain lesion-experiments the extent of lesion was determined by staining the sections with Nissl stain.



### I. *Control experiments for brain operations*

In this group 15 male dogs of 10 to 15 kg in weight were used. Cannulation of lumbo-adrenal vein was done 48 hours before the control brain operation. This operation includes the exposure of the brain after incision over the meninges through the trephine hole. The layers were closed as usual without any brain lesion. Burn trauma to the right hind limb was inflicted at 60 minutes after this procedure in five dogs, at 180 minutes in five dogs and at 24 hours in five dogs. Adrenal venous blood 17-OHCS output was measured at 1/2, 1, 2, 3 and 6 hours after burn. Then intravenous ACTH (0.8 unit/kg) was administered and the adrenocortical response was noted.

### II. *Decortication and burn*

Ten male dogs of 10 to 15 kg in weight were used in these experiments. The lumbo-adrenal vein was cannulated 48 hours before the brain operations. Bilateral decortication was carried out in a single stage. Bleeding was controlled by silver clips, gelfoam and ligation as required. Sixty minutes after decortication intravenous ACTH (0.8 unit/kg) was injected. Rest for 24 hours was allowed to the animals. Burn trauma to the right hind limb was applied and the adrenal venous 17-OHCS output was measured at 1/2, 1, 2 and 3 hours after burn. Intravenous ACTH test was repeated.

### III. *Animals with brain removed up to thalamo-hypothalamic level*

Five male dogs of 10.5 to 14 kg in weight were used in these experiments. Lumbo-adrenal vein was cannulated 48 hours before the brain removal operations. Removal of brain up to the thalamo-hypothalamic level was carried out on both sides. Posterior neural connections remained intact. At 30 and 60 minutes after these procedures adrenal venous blood 17-OHCS output was measured. Bleeding was checked as mentioned previously. Standardized burn trauma was inflicted on the right hind limb. Adrenal venous corticosteroid output was measured at 1/2, 1, 2 and 3 hours after burn. Intravenous ACTH was injected after this.

### IV. *Animals with solitary pituitary and intact hind brain*

In this group eight male dogs of 10 to 15 kg in weight were used. Lumbo-adrenal vein was cannulated 48 hours before the brain operations. The dogs were operated under nembutal anaesthesia. Brain removal was carried out through craniotomy openings by methods of section and suction.

During the process of removal of the brain, the middle cerebral arteries stand out in prominence and these require ligation to minimize haemorrhage. In all the experiments, both here and subsequently, where brain removal operations have been performed, bleeding from vessels has been checked by the following procedures as required :



(i) Ligations, (ii) application of Cushing's clips, (iii) cautery and (iv) gelfoam.

Supratentorial neural structures were removed leaving behind the pituitary only. The infratentorial structures were kept undisturbed. Adrenal venous blood was collected at 1/2, 1 and 3 hours after this procedure. Five per cent glucose and five per cent glucose in saline transfusions were carried out during the process of brain removal. The right inferior extremity was burned and corticosteroid output was measured at 1/2, 1, 2 and 3 hours after burn. ACTH (0.8 unit/kg) was administered intravenously. Artificial respiration was carried out in some dogs.

The blood-pressure changes during surgery are shown in Figs. 1-3.

The pituitary was histologically studied after staining the sections with Gomori's CAHP stain and haematoxylin and eosin stain.

#### V. *Absolutely solitary pituitary with removal of the hind brain*

Five male dogs of 10 to 15 kg in weight were used. The procedures were same as in group IV. Removal of infratentorial brain substance was an additional step. The tentorium cerebelli was removed and the cerebellum was extirpated and this facilitated the removal of the hind brain. The pituitary was left alone (solitary pituitary). These dogs required blood (recently drawn blood from hypophysectomized donors), fluid transfusions and artificial respiration. Sixty minutes after the brain operations standardized burn trauma was produced on the right hind limb and corticosteroid output was measured at 1/2, 1, 2 and 3 hours after burn. ACTH response was also noted.

Histological examination of the pituitary was carried out after staining the section with Gomori's CAHP stain and haematoxylin and eosin stain.

### RESULTS

#### I. *Control experiments for brain operations*

In this group adrenal venous 17-OHCS output was high during cannulation operation in comparison to the post-cannulation values (48 hours after cannulation).

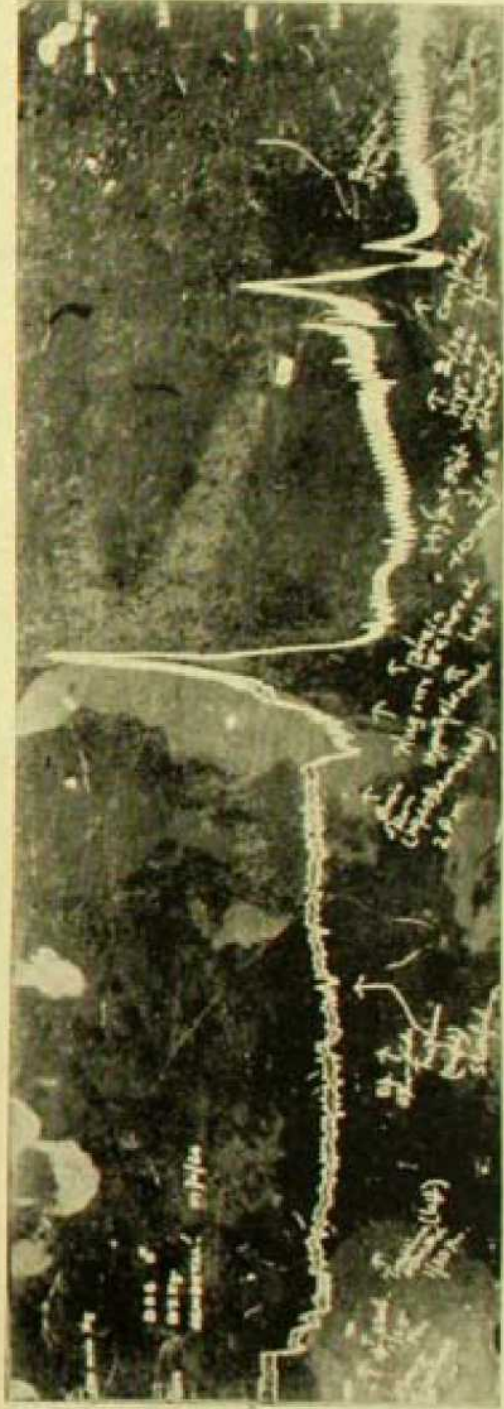
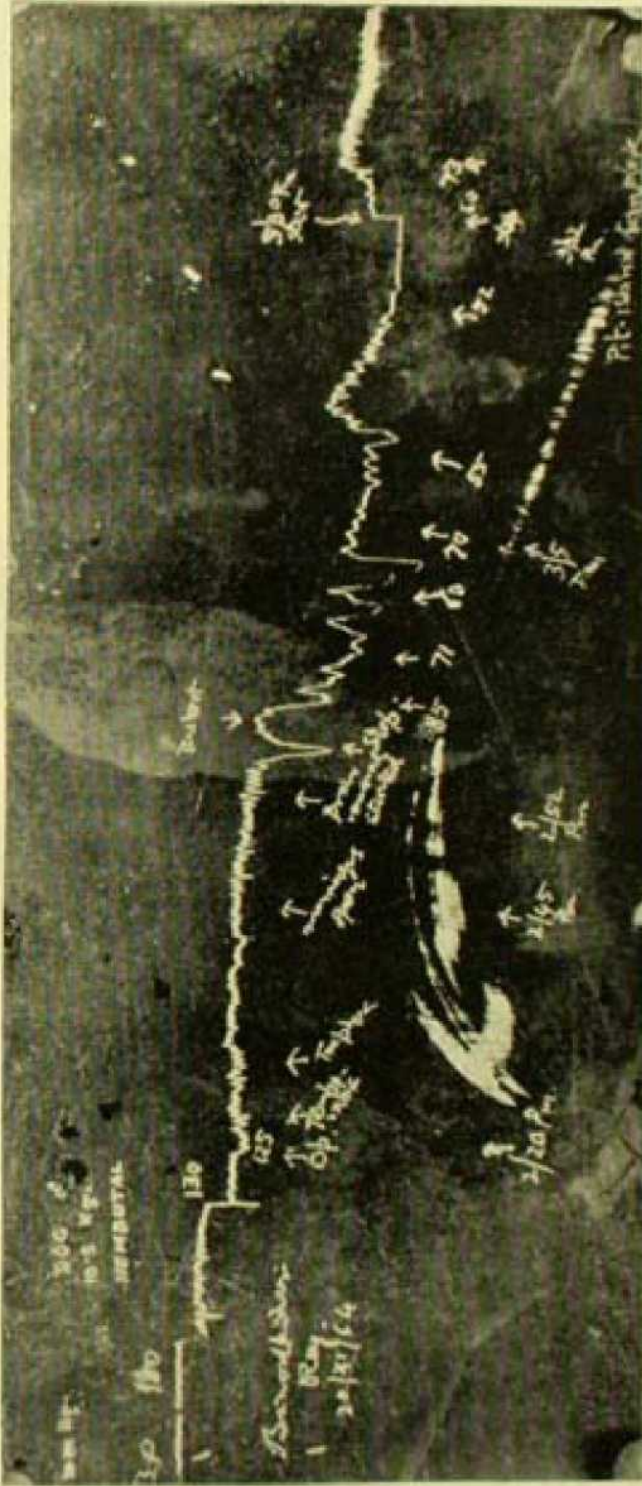
*Subgroup A* (Table I and Statistical Table I). During the surgery of control brain operations the corticosteroid output was significantly high at 30 and 60 minutes. Thirty minutes after burn the corticosteroid output was significantly high (0.1% level) when compared to the value at 60 minutes after brain operation. At 1, 2, 3 and 6 hours after burn the difference was significant at 1% level. Intravenous administration of ACTH showed good adrenocortical response six hours after burn.



TABLE I  
*Control experiments for the brain lesioned dogs and burn*

Dog numbers Observation numbers	137	138	139	140	141	Mean	S.D.	D.F.
	Adrenal venous 17-OHCS output (micrograms/minute)							
1. During adrenal vein cannulation	.. 8.2	7.8	9.5	10.1	11.0	9.320	1.326	4
2. Forty-eight hours after cannulation	.. 1.9	2.6	3.1	2.0	3.5	2.620	0.691	4
3. Thirty minutes post-operative	.. 9.5	10.2	11.6	12.0	12.5	11.160	1.262	4
4. Sixty minutes post-operative	.. 9.0	7.6	11.2	10.5	10.8	9.820	1.494	4
5. Thirty minutes after burn	.. 20.1	25.0	23.7	25.0	20.5	22.860	2.401	4
6. Sixty minutes after burn	.. 18.7	21.3	22.0	21.3	17.0	20.060	2.124	4
7. Two hours after burn	.. 17.2	22.3	20.0	18.5	16.4	18.880	2.349	4
8. Three hours after burn	.. 17.6	19.6	16.7	19.7	15.0	17.720	1.994	4
9. Six hours after burn	.. 15.3	18.5	17.4	16.6	15.6	16.680	1.314	4
10. ACTH (I.V.)	.. 21.0	24.8	21.5	24.0	22.7	22.800	1.611	4





FIGS. 1-2. 1, Blood pressure changes during surgery for isolated pituitary experiment;  
 2, blood-pressure changes during surgery for isolated pituitary experiment and born.



STATISTICAL TABLE I

Values of 't' together with D.F. for statistical tests of significance for different types of 'intra-group' paired comparisons regarding adrenal venous 17-OHCS output in dogs (control experiments for brain lesions)

				D.F.	't'
Observation	No.	1	vs.	Observation	No.
"	"	2	vs.	2	4
"	"	2	vs.	3	4
"	"	2	vs.	4	4
"	"	4	vs.	5	4
"	"	4	vs.	6	4
"	"	4	vs.	7	4
"	"	4	vs.	8	4
"	"	4	vs.	9	4
"	"	9	vs.	10	4
					12.727 †
					—18.852 †
					—11.861 †
					—9.674 †
					—8.470 *
					—5.992 *
					—5.700 *
					—6.558 *
					—10.426 †

\* Significant at 1% level.

† Significant at 0.1% level or more stringent level.

*Subgroup B* (Table II and Statistical Table II).—During control brain operations up to three hours the 17-OHCS output was significantly high in comparison to the 48 hours' post-cannulation value. Significantly high 17-OHCS output was noted up to six hours after burn and intra-venous ACTH injection showed good response.

STATISTICAL TABLE II

Values of 't' together with D.F. for statistical tests of significance for different types of 'intra-group' paired comparisons regarding adrenal venous 17-OHCS output in dogs (control experiments for the brain lesions)

				D.F.	't'
Observation	No.	1	vs.	Observation	No.
"	"	2	vs.	2	4
"	"	2	vs.	3	4
"	"	2	vs.	4	4
"	"	2	vs.	5	4
"	"	5	vs.	6	4
"	"	5	vs.	7	4
"	"	5	vs.	8	4
"	"	5	vs.	9	4
"	"	5	vs.	10	4
"	"	10	vs.	11	4
					10.897 †
					—20.189 †
					—10.261 †
					—31.584 †
					—19.123 †
					—8.608 *
					—10.876 †
					—18.154 †
					—8.757 †
					—4.607 *

\* Significant at 1% level.

† Significant at 0.1% level or more stringent level.



TABLE II  
*Control experiments for the brain lesioned dogs and burn*

Dog numbers Observation numbers	142	143	144	145	146	Mean	S.D.	D.F.
	Adrenal venous 17-OHCS output (micrograms/minute)							
1. During adrenal vein cannulation	9.5	8.7	9.6	10.5	7.1	9.080	1.278	4
2. Forty-eight hours after cannulation	3.0	2.4	2.6	1.5	1.9	2.280	0.589	4
3. Thirty minutes post-operative	10.7	11.0	12.6	10.2	9.6	10.520	1.128	4
4. Sixty minutes post-operative	10.3	12.4	9.5	10.0	7.5	9.940	1.756	4
5. Three hours post-operative	9.0	8.5	8.6	7.2	7.0	8.060	0.899	4
6. Thirty minutes after burn	21.8	22.7	24.5	20.1	18.8	21.580	2.210	4
7. Sixty minutes after burn	20.6	24.5	25.0	18.0	15.9	20.800	3.976	4
8. Two hours after burn	17.3	21.7	21.7	16.8	16.8	18.860	2.601	4
9. Three hours after burn	16.2	18.3	17.3	15.1	14.7	16.320	1.501	4
10. Six hours after burn	13.1	16.0	17.0	13.0	14.3	14.680	1.774	4
11. ACTH (I.V.)	24.6	20.1	22.5	19.6	18.3	21.020	2.513	4



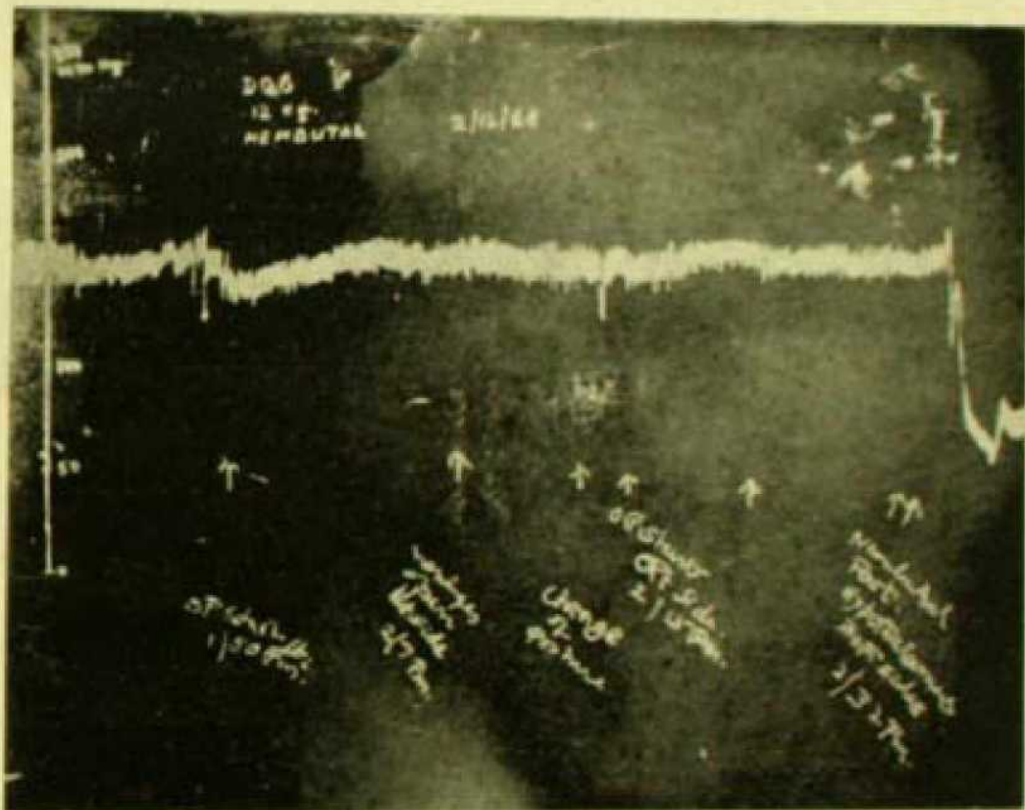
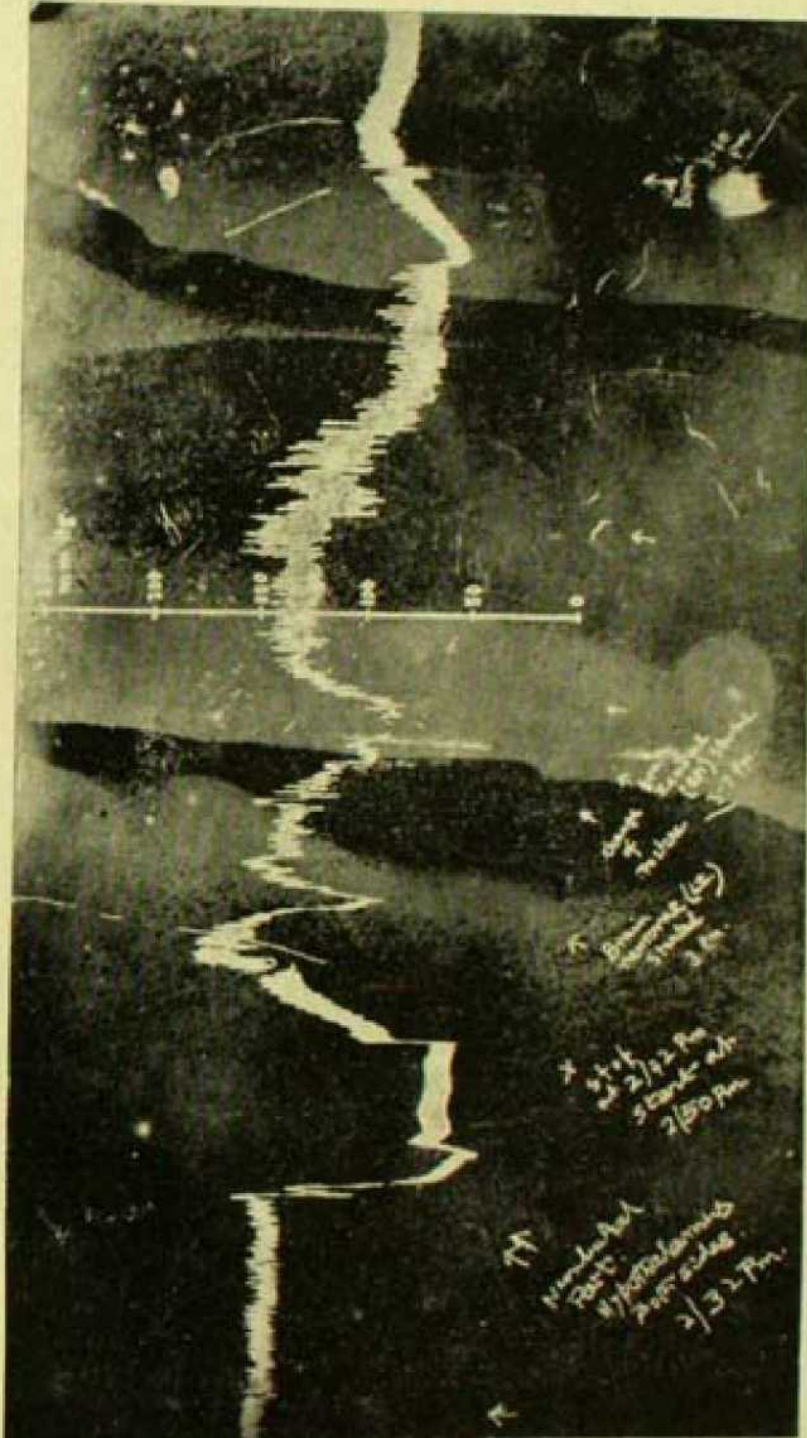


FIG. 3—Blood-pressure changes during surgery for isolated pituitary experiment and burn. Near the middle of the tracing, effect of injection of nembutal into the posterior hypothalamus on both sides is noted.







*Subgroup C* (Table III and Statistical Table III).—17-OHCS output was high up to six hours after control brain operations. After 24 hours the output reached a basal level. The corticosteroid output was high up to six hours after burn and intravenous ACTH administration showed a good response.

## II. Decortication experiments (Table IV and Statistical Table IV)

17-OHCS output reached a basal level 48 hours after cannulation. After 30 and 60 minutes of decortication the corticosteroid output was significantly high. This high output was also noted at 24 hours. Burn trauma stimulated the pituitary-adrenal-axis further and a good response was noted after intravenous ACTH.

STATISTICAL TABLE III

*Values of 't' together with D.F. for statistical tests of significance for different types of 'intra-group' paired comparisons regarding adrenal venous 17-OHCS output in dogs (control experiments for the brain lesions)*

				D.F.		't'
Observation	No.	1	vs.	Observation	No.	2
						4
						17.136 †
"	"	2	vs.	"	"	3
						4
						—11.424 †
"	"	2	vs.	"	"	4
						4
						—6.257 *
"	"	2	vs.	"	"	5
						4
						—7.024 *
"	"	2	vs.	"	"	6
						4
						—7.685 *
"	"	2	vs.	"	"	7
						4
						—2.749
"	"	7	vs.	"	"	8
						4
						—28.108 †
"	"	7	vs.	"	"	9
						4
						—22.272 †
"	"	7	vs.	"	"	10
						4
						—18.483 †
"	"	7	vs.	"	"	11
						4
						—25.125 †
"	"	7	vs.	"	"	12
						4
						—19.182 †
"	"	12	vs.	"	"	13
						4
						—7.351 *

\* Significant at 1% level.

† Significant at 0.1% level or more stringent level.

STATISTICAL TABLE IV

*Values of 't' together with D.F. for statistical tests of significance for different types of 'intra-group' paired comparisons regarding adrenal venous 17-OHCS output in dogs (decortication and burn)*

				D.F.		't'
Observation	No.	1	vs.	Observation	No.	2
						9
						15.900 *
"	"	2	vs.	"	"	3
						9
						—9.705 *
"	"	2	vs.	"	"	4
						9
						—9.267 *
"	"	2	vs.	"	"	6
						9
						—8.514 *
"	"	4	vs.	"	"	5
						9
						—9.730 *
"	"	6	vs.	"	"	7
						9
						—9.366 *
"	"	6	vs.	"	"	8
						9
						—9.097 *
"	"	6	vs.	"	"	9
						9
						—7.725 *
"	"	6	vs.	"	"	10
						9
						—5.641 *
"	"	10	vs.	"	"	11
						9
						—10.984 *

\* Significant at 0.1% level or more stringent level.



TABLE III  
*Control experiments for the brain lesioned dogs and burn*

Dog numbers Observation numbers	147	148	149	150	151	Mean	S.D.	D.F.
	Adrenal venous 17-OHCS output (micrograms/minute)							
1. During a renal vein cannulation	.. 10.0	9.4	9.5	8.5	10.7	9.620	0.811	4
2. Forty-eight hours after cannulation	.. 1.3	2.5	2.7	2.0	2.5	2.200	0.566	4
3. Thirty minutes post-operative	.. 11.4	10.7	9.9	8.0	11.5	10.300	1.438	4
4. Sixty minutes post-operative	.. 11.6	11.0	6.5	7.5	10.0	9.320	2.222	4
5. Three hours post-operative	.. 10.5	7.1	7.2	8.4	10.6	8.760	1.713	4
6. Six hours post-operative	.. 8.0	6.5	6.0	7.0	6.4	6.780	0.769	4
7. Twenty-four hours post-operative	.. 5.2	3.6	3.5	2.9	3.8	.800	0.852	4
8. Thirty minutes after burn	.. 23.0	20.7	21.6	23.3	24.0	22.520	1.341	4
9. Sixty minutes after burn	.. 21.9	18.1	18.5	17.8	22.1	19.680	2.134	4
10. Two hours after burn	.. 21.0	15.4	18.0	19.0	17.5	18.180	2.052	4
11. Three hours after burn	.. 18.2	16.4	17.2	18.6	18.7	17.820	0.981	4
12. Six hours after burn	.. 18.7	13.5	14.5	14.7	16.2	15.520	2.023	4
13. ACTH (I.V.)	.. 21.0	18.6	20.3	20.1	22.1	20.420	1.283	4





TABLE IV  
*Decortication and burn*

Dog numbers Observation numbers	..	..	..	157	158	159	160	161	162	163	164	165	166	Mean	S.D.	D.F.
Adrenal venous 17-OHCS output (micrograms/minute)																
1. During adrenal vein cannulation	..	..	..	12.4	8.4	7.5	10.0	9.2	8.6	10.0	9.0	11.5	7.4	9.400	1.619	9
2. Forty-eight hours after cannulation	..	..	..	3.2	2.4	2.7	3.1	2.8	1.9	2.6	3.5	3.0	2.7	2.690	0.567	9
3. Thirty minutes after decortication	..	..	..	22.6	12.0	16.9	11.7	13.0	8.1	18.5	14.0	18.2	13.5	14.850	4.539	9
4. Sixty minutes after decortication	..	..	..	18.0	10.2	19.4	13.5	12.0	11.5	23.0	15.2	23.0	20.0	16.580	4.749	9
5. ACTH (I.V.)	..	..	..	27.1	18.5	23.1	27.2	20.0	19.5	32.1	28.0	34.0	26.5	25.600	5.264	9
6. 24 hours after decortication	..	..	..	14.4	9.2	12.2	10.1	9.3	8.5	15.0	12.0	20.0	16.5	12.720	3.725	9
7. Thirty minutes after burn	..	..	..	30.1	19.0	20.0	25.0	22.1	16.7	34.0	29.0	28.6	27.5	25.200	5.579	9
8. Sixty minutes after burn	..	..	..	24.0	20.5	18.1	19.0	16.5	12.5	27.1	18.4	28.7	21.0	20.580	4.895	9
9. Two hours after burn	..	..	..	24.6	17.6	18.4	15.9	14.0	16.1	30.5	21.2	26.0	21.7	20.600	5.212	9
10. Three hours after burn	..	..	..	18.4	14.1	14.5	12.0	13.5	14.0	26.7	18.5	26.6	19.5	17.780	5.287	9
11. ACTH (I.V.)	..	..	..	30.0	23.0	20.5	22.0	24.7	26.6	35.2	28.0	32.1	25.0	26.710	4.656	9



When the results at 60 minutes after decortication were compared to those of the control brain operations (Statistical Table XI), the difference was significant at 1% level, the decortication group manifested with higher adrenocortical activity. At 24 hours the difference was significant at 0.1% level. By this time the corticosteroid output reached a basal level in the dogs with control brain operations, but the decorticated dogs had high 17-OHCS output. Regarding the responses after burn trauma there was practically no difference between the two groups.

### III. Animals with brain removal up to thalamo-hypothalamic level (Table V and Statistical Tables V and VIII)

TABLE V

*Brain removal down to thalamo-hypothalamic level (posterior connections remaining intact) and burn*

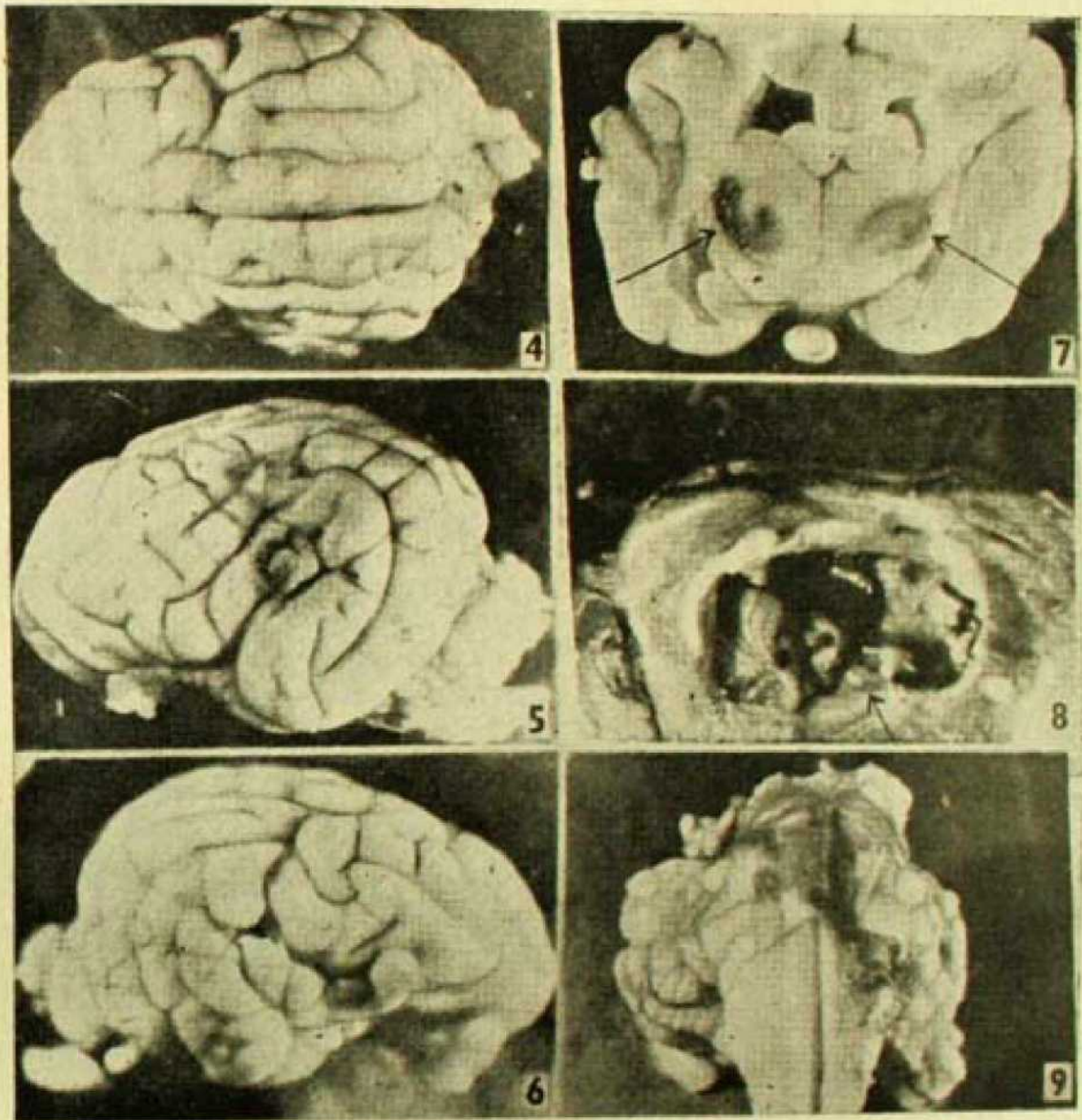
Dog numbers	..	..	..	167	168	169	170	171	Mean	S.D.	D.F.
Observation numbers	Adrenal venous 17-OHCS output (micrograms/minute)										
1	During adrenal vein cannulation	..	..	11.2	13.0	7.0	10.4	7.4	9.800	2.557	4
2	Forty-eight hours after cannulation	..	..	3.1	2.7	1.9	2.6	2.0	2.460	0.503	4
3	Thirty minutes after the preparation	..	..	7.1	1.5	6.4	7.0	5.0	5.400	2.336	4
4	Sixty minutes after the preparation	..	..	11.5	1.2	6.0	10.5	6.4	7.120	4.106	4
5	Thirty minutes after burn	..	..	30.6	8.5	21.5	26.8	23.2	22.120	8.380	4
6	Sixty minutes after burn	..	..	25.0	13.4	17.8	20.2	18.0	18.880	4.218	4
7	Two hours after burn	..	..	20.4	12.7	19.0	14.5	14.1	16.140	3.355	4
8	Three hours after burn	..	..	17.0	13.0	22.0	18.2	21.3	18.300	3.622	4
9	ACTH (I.V.)	..	..	18.0	16.6	26.9	24.2	26.4	22.420	4.809	4

Basal 17-OHCS output was noted 48 hours after cannulation. Surgery for removal of the brain above the hypothalamic level did not show significantly high 17-OHCS output. However, when burn trauma was superimposed, increased 17-OHCS output was observed and the adrenal cortex was well responsive to exogenous ACTH.

Thirty minutes after control brain operation the corticosteroid output was significantly higher than in the group of dogs with lesions up to thalamo-hypothalamic level. At no other periods there was any significant difference.

In a few experiments when only bilateral thalamic lesions were produced, burn trauma to the right hind limb (on the same day) did not manifest with immediate increased 17-OHCS output (Figs. 4, 5, 6 and 7 of the same dog).





FIGS. 4-9. 4, dorsal view of the brain. 5, left lateral view of the same brain as of Fig 4. 6, right lateral view of the same brain as of Fig. 4. 7, shows the bilateral thalamic lesions of the same brain. 8, shows the solitary pituitary and the intact hind brain within the calvarium of dog No. 172. 9, ventral view of the infratentorial structures of dog No. 172.



#### IV. Animals with solitary pituitary and intact hind brain (Table VI and Statistical Tables VI and X)

Forty-eight hours after cannulation the basal 17-OHCS output was noted. The solitary pituitary with intact infratentorial structures responded well to surgery at 30, 60 and 180 minutes. Superadded burn trauma manifested with high 17-OHCS output up to three hours and the adrenal cortex was well responsive to exogenous ACTH.

STATISTICAL TABLE V

Values of 't' together with D.F. for statistical tests of significance for different types of 'intra-group' paired comparisons regarding adrenal venous 17-OHCS output in dogs (brain removal down to thalamo-hypothalamic level—posterior connections remaining intact and burn)

								D.F.	't'
Observation	No.	1	vs.	Observation	No.	2	4		7.565 *
"	"	2	vs.	"	"	3	4	—	2.753
"	"	2	vs.	"	"	4	4	—	2.630
"	"	4	vs.	"	"	5	4	—	7.440 *
"	"	4	vs.	"	"	6	4	—	19.216 †
"	"	4	vs.	"	"	7	4	—	5.767 *
"	"	4	vs.	"	"	8	4	—	5.526 *
"	"	8	vs.	"	"	9	4	—	4.741 *

\* Significant at 1% level.

† Significant at 0.1% level or more stringent level.

STATISTICAL TABLE VI

Values of 't' together with D.F. for statistical tests of significance for different types of 'intra-group' paired comparisons regarding adrenal venous 17-OHCS output in dogs (solitary pituitary, hind brain and burn)

								D.F.	't'
Observation	No.	1	vs.	Observation	No.	2	7		17.089 †
"	"	2	vs.	"	"	3	7	—	3.528 *
"	"	2	vs.	"	"	4	7	—	3.683 *
"	"	2	vs.	"	"	5	7	—	6.810 *
"	"	5	vs.	"	"	6	7	—	11.198 †
"	"	5	vs.	"	"	7	7	—	6.081 †
"	"	5	vs.	"	"	8	7	—	5.908 †
"	"	5	vs.	"	"	9	7	—	7.289 †
"	"	9	vs.	"	"	10	7	—	5.958 †

\* Significant at 1% level.

† Significant at 0.1% level or more stringent level.







STATISTICAL TABLE VII

Values of 't' together with D.F. for statistical tests of significance for different types of 'intra-group' paired comparisons regarding adrenal venous 17-OHCS output in dogs (all brain including hind brain ablated—absolutely solitary pituitary and burn)

				D.F.				't'
Observation	No.	1	vs.	Observation	No.	2	4	9.115 ‡
"	"	2	vs.	"	"	3	4	1.666
"	"	2	vs.	"	"	4	4	1.668
"	"	4	vs.	"	"	5	4	4.316 *
"	"	4	vs.	"	"	5	4	10.385 ‡
"	"	4	vs.	"	"	7	4	5.709 †
"	"	4	vs.	"	"	8	4	7.158 †
"	"	8	vs.	"	"	9	4	5.700 †

\* Significant at 5% level.

† Significant at 1% level.

‡ Significant at 0.1% level or more stringent level.

STATISTICAL TABLE VIII

Showing D.F. and values of 't' and their significance for different types of 'intergroup' comparisons

Table I			vs.	Table V			D.F.	't'
Observation	No.	3	vs.	Observation	No.	3	8	4.853 *
"	"	4	vs.	"	"	4	8	1.382
"	"	5	vs.	"	"	5	8	0.190
"	"	6	vs.	"	"	6	8	0.559
"	"	7	vs.	"	"	7	8	1.496
"	"	8	vs.	"	"	8	8	-0.314

\* Significant at 1% level.

STATISTICAL TABLE IX

Showing D.F. and values of 't' and their significance for different types of 'intergroup' comparisons

Table I			vs.	Table VII			D.F.	't'
Observation	No.	3	vs.	Observation	No.	3	8	1.224
"	"	4	vs.	"	"	4	8	0.095
"	"	5	vs.	"	"	5	8	1.902
"	"	6	vs.	"	"	6	8	-0.482
"	"	7	vs.	"	"	7	8	0.073
"	"	8	vs.	"	"	8	8	0.294



Out of the eight dogs in this group two dogs (Dog numbers 174 and 177) showed low 17-OHCS output at 30 and 60 minutes after the brain operation.

Comparison of the results of this group with those of the control brain operation-group showed significant difference only at 30 minutes.

Photographs of the brain lesions after solitary pituitary experiments have been presented in figure numbers 8, 9, 10, 11 (dog number 172), 12, 13, 14 (dog number 176) and 15, 16, 17 (dog number 178).

Histologically the pituitary did not show any gross change and there was no evidence of any serious vascular jeopardization.

V. *Absolutely solitary pituitary with removal of the hind brain* (Table VII and Statistical Tables VII and X)

STATISTICAL TABLE X

*Showing D.F. and values of 't' and their significance for different types of 'intergroup' comparisons*

Table II				vs.	Table VI				D.F.	't'
Observation	No.	3	vs.	Observation	No.	3	11			
"	"	4	vs.	"	"	4	11			2.841 *
"	"	5	vs.	"	"	5	11			1.612
"	"	6	vs.	"	"	6	11			-0.314
"	"	7	vs.	"	"	7	11			0.226
"	"	8	vs.	"	"	8	11			1.372
"	"	9	vs.	"	"	9	11			0.861
"	"			"	"					0.462

\* Significant at 5% level.

STATISTICAL TABLE XI

*Showing D.F. and values of 't' and their significance for different types of 'intergroup' comparisons*

Table III				vs.	Table IV				D.F.	't'
Observation	No.	3	vs.	Observation	No.	3	13			-2.152
"	"	4	vs.	"	"	4	13			-3.202 *
"	"	7	vs.	"	"	6	13			-5.195 †
"	"	8	vs.	"	"	7	13			-1.041
"	"	9	vs.	"	"	8	13			-0.387
"	"	10	vs.	"	"	9	13			-0.985
"	"	11	vs.	"	"	10	13			-0.016

\* Significant at 1% level.

† Significant at 0.1% level or more stringent level.



The high adrenal venous 17-OHCS output reached a basal level 48 hours after cannulation. The corticosteroid output at 30 and 60 minutes after absolute isolation of the pituitary was not significantly high in comparison to the value noted at 48 hours after cannulation and, moreover, in 2 dogs (dog numbers 180 and 183) there was practically no tendency for the corticosteroid output to rise. No rest period was allowed and in the post-burn period there was significantly high level of corticosteroid output. The adrenals were well responsive to exogenous ACTH.

TABLE VII

*All brain including hind brain ablated (absolutely solitary pituitary) and the effect of burn*

Dog numbers Observation numbers			180	181	182	183	184	Mean	S.D.	D.F.
			Adrenal venous 17-OHCS output (micrograms/minute)							
1	During adrenal vein cannulation	..	13.1	10.0	14.5	11.6	7.0	11.240	2.904	4
2	Forty-eight hours after cannulation	..	4.5	2.9	3.8	2.7	1.6	3.100	1.107	4
3	Thirty minutes after the preparation	..	0.9	12.5	10.6	2.5	13.1	7.920	5.780	4
4	Sixty minutes after the preparation	..	1.5	16.0	10.5	1.4	18.0	9.480	7.828	4
5	Thirty minutes after burn	..	4.2	20.5	19.0	3.9	24.4	14.400	9.652	4
6	Sixty minutes after burn	..	11.2	28.1	27.0	14.7	28.5	21.900	8.282	4
7	Two hours after burn	..	8.7	20.7	24.5	10.0	29.0	18.580	8.935	4
8	Three hours after burn	..	7.0	25.0	24.3	10.5	28.2	19.000	9.552	4
9	ACTH (I.V.)	..	12.1	29.6	26.2	14.0	30.6	22.500	8.805	4

From the scrutiny of Table VII it appears that dog numbers 180 and 183 show results different from those shown by dog numbers 181, 182 and 184 at every stage of experiment after the brain operation. In order to examine whether the subset consisting of dog numbers 180 and 183 differ from the subset consisting of dog number 181, 182 and 184, statistical tests have been applied. The values of Fisher's 't' for the comparisons of the two subsets are given on next page.



Observation numbers	Mean and S.D. of dog Nos. 180 and 183	Mean and S.D. of dog Nos. 181, 182, 184
	Subset 1	Subset 2
3	1.70 (1.131)	12.07 (1.305)
4	1.45 (0.071)	14.83 (3.884)
5	4.05 (0.212)	21.30 (2.787)
6	12.95 (2.475)	27.87 (0.777)
7	9.35 (0.919)	24.43 (4.155)
8	8.75 (2.475)	25.83 (2.079)
9	13.05 (1.344)	28.80 (2.311)

Observation numbers	Subset 1 vs. Subset 2	
	D.F.	't'
3 vs. 3	3	9.086 †
4 vs. 4	3	4.630 *
5 vs. 5	3	8.289 †
6 vs. 6	3	10.453 †
7 vs. 7	3	4.907 *
8 vs. 8	3	8.432 †
9 vs. 9	3	8.472 †

Histologically the pituitaries did not show any gross change.

\* Significant at 5% level.

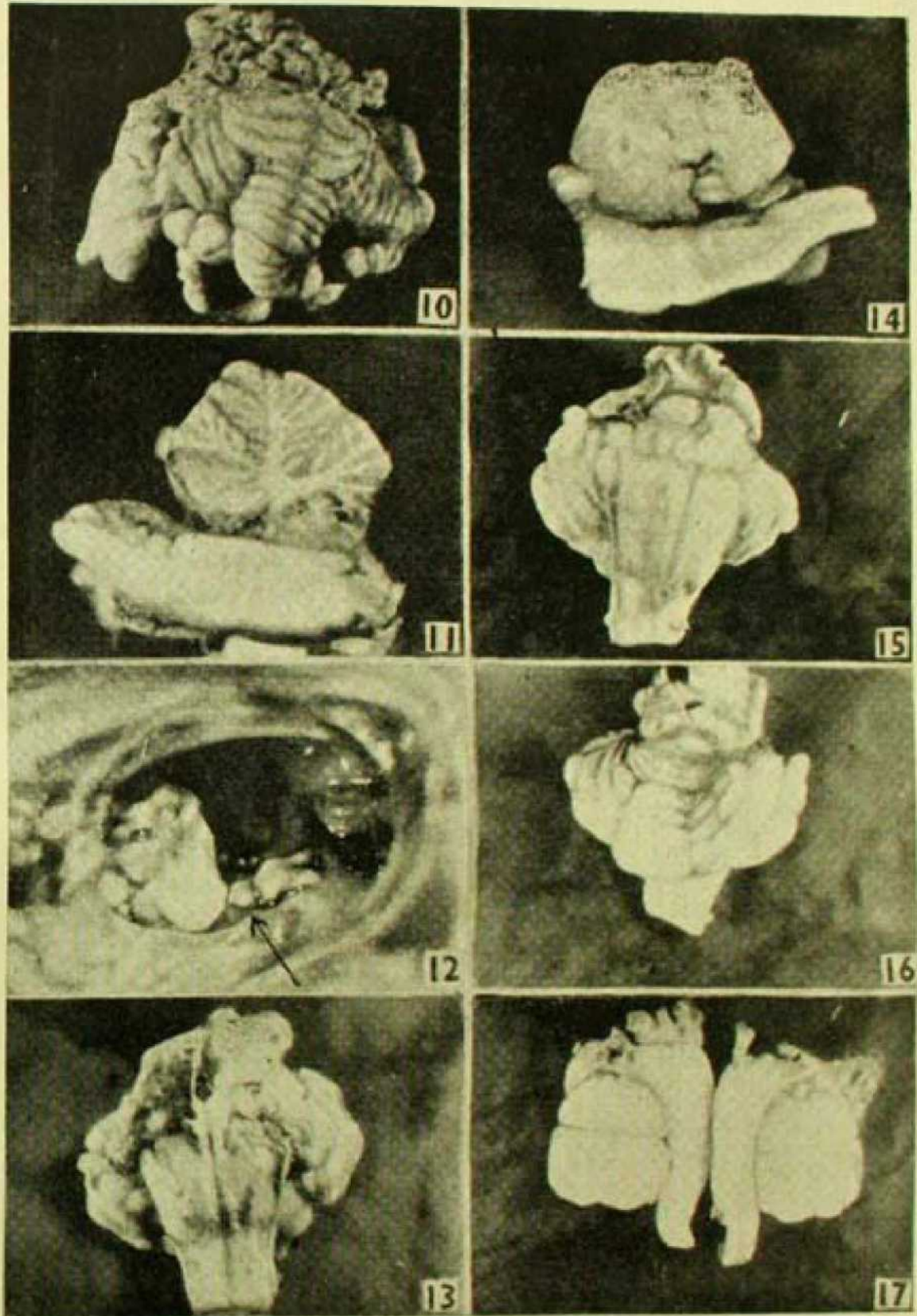
† Significant at 1% level.

## DISCUSSION

In the control brain operation groups low 17-OHCS outputs are noted at 24 and 48 hours after such operations. The corticosteroid output is high during and after such operations. In all cases there is increased adrenocortical response after burns. ACTH response is good in these experiments.

The cerebral cortex of the dog has an inhibitory influence over the centrally located structures responsible for ACTH release, because 24 hours





FIGS. 10—17. 10, dorsal view of the infratentorial structures of dog No. 172. 11, midline sagittal section of the infratentorial structures of dog No. 172. 12, shows the solitary pituitary of dog No. 176. 13, ventral view of infratentorial structures of dog No. 176. 14, mid-sagittal section of the infratentorial structures of dog No. 176. 15, ventral view of infratentorial structures of dog No. 178. 16, dorsal view of infratentorial structures of dog No. 178. 17, mid-sagittal section of infratentorial structures of dog No. 178.



after decortication the 17-OHCS output is significantly higher than that of the control brain operation-group. That the increased level at this period is not due to the stress of surgery only is proved by the low level of 17-OHCS output in the control brain operation-group. Burn trauma and ACTH manifested with significantly increased activity of the pituitary adrenal axis. Roy (1962) mentioned about this cortical inhibition. Egdahl (1961*b*) discussed about the cerebral cortical inhibition of the pituitary adrenal secretion. He used sciatic nerve stimulation in the anaesthetized decorticated animal as a form of stress. With this stress there was a significant adrenal cortical response. There was no further increase in corticosteroid output after exogenous ACTH. However, in the present investigation increased response to exogenous ACTH has been noted.

There is a species difference in the adrenocortical response after bilateral decortication. Matsuda *et al.* (1963) did not observe any cerebral cortical inhibition in the rat as in these animals there was no sustained rise in peripheral plasma corticosteroid level after removal of the cerebral cortex and subjacent brain.

Setekleiv *et al.* (1961) observed increase in plasma 17-hydroxycorticosteroids by cerebral cortical and amygdaloid stimulation in the cat. There was no significant increase on stimulation through 21 cortical electrodes on the lateral surface of the brain. The position of the electrodes were mainly in the frontal and parietal lobes including sensory-motor cortex and the orbital gyrus. The middle and the posterior cingulate areas were also negative regarding the response.

Adrenocortical response to burn trauma is more in dogs having the hypothalamus intact (brain removed upto thalamohypothalamic level).

In the solitary pituitary experiments with the infratentorial structures intact, high 17-OHCS output has been observed and burn stimulates the same axis further. Egdahl (1960) thought that the hind brain factor could pass through the systemic circulation and act on the isolated pituitary. Roy (1960) observed similarly and thought that when the inhibitory influences from higher centres were removed, the pituitary could activate in a more pronounced way. Possibilities of infratentorial neurosecretion and histamine release at the injured site were also thought of. The pituitary gland is very labile in such conditions and any chemical alteration or pH change in blood will lead to increased ACTH discharge. Roy (1962) found that zinc can stimulate the infratentorial neurosecretion which could pass via the systemic circulation and act on the solitary pituitary. Moreover, in such a preparation, as the inhibitory influence of the neocortex and hippocampus was lost, the basal value of 17-OHCS output was increased.

Subsequently it has been observed by Wise *et al.* (1962) and Egdahl (1962) that the pituitary can respond even in the absence of the hind brain. Removal of the total brain and spinal cord or removal of posterior pituitary or abdominal viscera does not change the response (Egdahl 1962). In the present investigation removal of the infratentorial structures cannot block the raised 17-OHCS output in response to burn trauma, proving thereby that the HBF is not important for the increased ACTH response after stress. When the infratentorial structures are removed, there is



low 17-OHCS output upto 60 minutes after the preparation, but such dogs respond to burn trauma with increased 17-OHCS output. In a total brainless dog the pituitary has only systemic circulation and any fluctuation in the ACTH secretion is solely due to substances released in circulation. The neurosecretion and chemicals from the sympathetics may be an answer to this problem, but we have seen increased adrenocortical response in partially sympathectomized and splanchnicectomized dogs with total ablation of central nervous system structures after burns. Moreover, Egdahl (1962) has shown that posterior pituitary, kidneys or other abdominal organs are not responsible for the adrenocortical secretory status of these animals. Gann *et al.* (1961) thought of an ACTH releasing substance produced by the kidney in dogs with isolated pituitaries.

What we surgically perform in solitary pituitary experiments is seen in nature-made anencephaly. In the latter condition there is a fundamental defect in the formation of the brain and so there is hypoplasia of the secretory elements of the pituitary which is reflected over the ductless glands.

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## SECTION B

### BRAIN MECHANISMS RESPONSIBLE FOR ACTH RELEASE IN EXPERIMENTAL BURNS (1967)

Maximum adrenocortical response after burn trauma appears late in dogs with lesion of the anterior hypothalamus. Adrenocortical response to burn trauma in such dogs is of lesser magnitude when the comparison is made with the response in dogs having the hypothalamus intact (brain removed upto thalamohypothalamic level). Inhibitory influences over the pituitary-adrenocortical secretion are exerted by the hippocampus, the septum, and the cingulate area. Dogs with lesions of any of these areas show increased adrenocortical activity and burn trauma enhances the response further. Balancing action is exerted by the cingulate area between the hippocampus and the hypothalamus regarding the pituitary-adrenocortical activity. Restraining influence over the pituitary-adrenocortical activity seems to be played by the amygdala and this may be due to the associated vascular lesion of the hippocampus. Dogs with amygdaloid lesion show increased 17-OHCS output in response to burn trauma. Adaptation activity also occurs at the brain level through different stimulatory and depressive areas of the brain and their afferent and efferent fibre systems. The net result is a steady state in the body in adverse situations what Claude Bernard has said long time back. Thus apart from the endocrinal adaptation, the central neural integrations are also to be considered.

## INTRODUCTION

Different brain areas control the activity of the hypothalamo-pituitary-adrenal-axis. In the present investigation the controlling authorities have been studied in relation to burn trauma by ablation techniques.

Spiegel *et al.* (1940) studied the parts of the forebrain related to the starting of the rage reactions in decorticate cats and dogs. There was no rage reaction after lesions of the neocortical areas. It appeared when the extirpation of the frontal lobes encroached upon the olfactory tubercles or there were isolated lesions of the tubercles. After lesions of the hippocampus—fornix system there were rage reactions when the fornical lesions encroached upon the septum pellucidum. Bilateral lesions of amygdaloid nuclei definitely led to outbursts of rage.

Bard and Mountcastle (1948) studied some forebrain mechanisms involved in expression of rage with special reference to suppression of angry behaviour in cats. They found no evidence to support the view that



ablation of forebrain structures, either neocortical or rhinencephalic, situated in front of the optic chiasma lead to lowering of threshold of rage reactions. They found that bilateral removal of amygdaloid nuclei produced marked outbursts of angry behaviour. Klüver and Bucy (1939) and Bucy and Klüver (1940) observed that after bilateral ablations of temporal lobes, the greater part of hippocampus, uncus, and amygdala, the fear reactions of wild *Macaca mulatta* were reduced to a great extent. These authors (Bucy and Klüver, 1955; Klüver, 1952; Klüver and Bartelmez, 1951; Klüver and Bucy, 1937, 1938, 1939) described the following syndrome in monkeys after bilateral anterior temporal lobectomy and amygdalectomy: (a) visual agnosia, (b) hypermetamorphosis, (c) oral compulsive behaviour, (d) marked change in emotional behaviour with fearlessness and aggressiveness, (e) hypersexuality, (f) dietary habits are changed with acceptance of meat as food, and (g) development of increased and peculiar spontaneous motor activities.

In cats bilateral amygdalectomy produces placidity (Morin *et al.*, 1952; Schreiner and Kling, 1953a, 1953b, and 1954). Thus there are two different opinions regarding the behavioural change after bilateral amygdalectomy. Schreiner and Kling (1953b and 1954) tried to explain this discrepancy in carnivores on the basis of hypersexuality as the savage behaviour disappeared after castration. Green (1960) thought that savage behaviour was produced by the interference of blood supply to the anterior hippocampus. Placidity was produced by vascular disturbances in basal ganglia.

Fulton (1951) in his lecture on "Frontal lobotomy and affective behaviour" said that lesions of the various components of the visceral brain-hippocampus, amygdala, insula, cingulate, temporal tip and posterior orbital gyrus produced autonomic disturbances, and there were behavioural changes in the form of placidity. However, isolated lesions of amygdala in cats produced ferocity.

### Limbic System and Adrenocortical Response

Porter (1954) found in the brain of monkeys (*Macaca mulatta*) both stimulatory and inhibitory areas for the activation of the pituitary-adrenal-axis. An area in the cerebral cortex was found which, when stimulated gave rise to eosinopenia. The maximum response was noted after excitation of the frontal lobe and particularly the orbital surface. Stimulation of the surrounding prefrontal cortex also led to significant eosinopenia. He noted an inhibitory area in the hippocampal region. Stimulation of the uncus reduced the eosinopenic response to epinephrine (intravenously) or operative trauma. Excitation of the surrounding hippocampal region reduced the response to less than 50% of the controls. Porter further said that the amygdaloid nucleus was studied but no eosinopenia was noted.

Endrőczy and Lissák (1953) stated that after removal of the frontal cortex the sensitivity to different stresses became different. The altered response must be ascribed to changes in neural mechanism.





Endröczy *et al.* (1954) observed that surgical interventions involving the neocortical structures did not show much change in the pituitary-adrenocortical system when assessment was made by noting the blood chemistry and the histochemical changes. Cortin-like substance in the blood was within normal limits. Cats with lesions of the rhinencephalic structures showed marked rage response to both spontaneous and slight stimuli. The blood glucocorticoid level was very high. The histochemistry of the adrenal cortex corroborated the changes noted in the cortin-like substances in the plasma.

Woods (1956) described the taming of the wild Norway rat by rhinencephalic lesions. Damage to the brain in the area of the amygdala produces an immediate and in most cases permanent loss of fierceness and aggressiveness in wild Norway rats. In no brain the amygdala was completely destroyed and in all cases the lesion was restricted to rhinencephalic structures.

Woods (1957) studied stress reaction in wild and domesticated rats. In wild rats there was no change in ascorbic acid and lipid content of the adrenal cortex after stress (temperature and emotional stimulation), but changes were noted same after ACTH as in the domesticated rats.

Martin *et al.* (1958) observed elevation of adrenocortical secretion rate after removal of amygdalic nuclei.

Mason (1959) observed a rise in plasma 17-OHCS level in monkeys after amygdaloid stimulation.

Emotional arousal (anxiety and anger) gives rise to an increased output of adrenocortical hormones in man (Persky *et al.*, 1958 ; Skaug, 1960), and also in animals (Mason *et al.*, 1957a ; Mason *et al.*, 1957b ; and Skaug, 1960).

Ganong and Goldfien (1959) noted rise in plasma 17-OHCS after stimulation of the orbital surface of the frontal lobe in the dog.

Kim and Kim (1961) observed that the hippocampus exerts a sustained inhibitory influence upon the pituitary-adrenocortical mechanism in rats.

Setekleiv *et al.* (1961) studied plasma 17-OHCS by cerebral cortical and amygdaloid stimulation in the cat. Behavioural arousal and fear could be induced by electrical stimulation of the cingulate, temporo-occipital cortex and the amygdala in the cat. Rise in plasma 17-OHCS level was noted after stimulation of the anterior cingulate cortex, the lower portion of the posterior ecto-and suprasylvian gyri of the temporo-occipital cortex and the amygdala. The response was obtained during light anaesthesia.

Anand *et al.* (1957) reported that the eosinopenic responses to stress (subcutaneous injection of hypertonic saline) were same before and after frontal and temporal lesions. This indicates according to them that the regions thus ablated do not take part in the secretion of ACTH after stress.

Anand (1963) discussed the functional importance of the limbic system. He mentioned that ablation and stimulation studies on the limbic structures did not show any changes in ACTH secretion.



Watson (1907) and King and Donaldson (1929) found the adrenals of recently caught wild rats to be bigger than those of the laboratory animals. Hatai (1915) and Donaldson (1923) mentioned this to be due to a special cortical enlargement in the wild variety. Emery (1935) noted bigger adrenals in a family of hairless rats derived from a wild ancestry in comparison to those of the albino rats. According to Hatai (1915) the adrenals of the wild rats (*R. norvegicus*) were almost doubly heavier than those of the albino rats. Rogers and Richter (1948) noted heavier adrenals in the wild rats. Richter *et al.* (1950) found that after adrenalectomy wild rats could survive only in 2% of cases with salt therapy. On the other hand 87% of the domesticated rats, after the same procedure could survive for a longer period with salt therapy. Accessory cortical tissue was present in the wild rats in 11.3% of cases; whereas it was 4.3% in domesticated rats. Thus there is a difference between the wild and domesticated rats regarding the nature of the adrenocortical function.

Increased adrenocortical function has been noted after stimulation of the posterior orbital surface by Endrőczy *et al.* (1958) and Okinaka *et al.* (1960).

Stimulation of the hippocampus in different types of animals checks the functions of the pituitary-adrenocortical system. The function of the above system was judged by the number of lymphocytes in rabbits and dogs, the adrenal ascorbic acid content in rats and the corticoid content of the adrenal venous blood in cats. Suppressing activity of the hippocampus on the pituitary-adrenal system was noted. There was a considerable check in lymphopaenia and change in ascorbic acid content of the adrenal and the corticoid secretion after stimulation of archicortical structures. Lissák and Endrőczy (1960) could prove that the rostral part of the caudate nucleus when stimulated led to suppression. Thus the checking region is not a circumscribed structure but it is a complex mechanism where the archicortex takes the leading role. Amygdaloid stimulation manifested with increased secretion in cats. They also noted stimulation of the pituitary-adrenal-axis after amygdalectomy.

Karli (1956) noted that the aggressivity of wild rats was very much diminished after amygdalectomy. In the "mouse-murder" test 70% of the wild rats killed the mice instantaneously. Amygdalectomy completely abolished it. Aggressivity, however, appeared in domesticated rats after frontal lobectomy. Lissák and Endrőczy (1960) said that the influence of amygdala upon behaviour and its relationship with the pituitary-adrenocortical function played an important part probably also in the adaptation under normal conditions. They could elicit fall in ascorbic acid content of adrenals in rats after stimulating the different nuclear groups in thalamus (dorso-medial and ventro-medial).

Endrőczy *et al.* (1959) described the inhibitory influence of archicortical structures on pituitary-adrenal function. In the dog, cat, rabbit and rat the amygdaloid nucleus was stimulated by permanent deep electrodes. This resulted in marked increase of pituitary-adrenocortical activity. Stimulation of hippocampus, however, inhibited the increase in ACTH secretion by the anterior pituitary after painful electric shock, and injection



of adrenaline, histamine or formalin. Assessment of the adrenocortical function was done by measuring ascorbic acid content of the adrenals, the corticoid content of the adrenal venous blood and by the lymphopenic response test.

Endrőczi and Lissák (1960) stimulated the reticular formation and posterior hypothalamus of cats and dogs and found changes in adrenocortical secretion similar to those observed after chronic ACTH treatment. Stimulation of the pyriform lobe and the amygdaloid nucleus led to sexual behaviour and to the secretion of gestagenic and androgenic substances (11- and 17-hydroxyprogesterone, respectively).

Endrőczi and Lissák (1962) said that the influence of hippocampal stimulation on the pituitary-adrenocortical system of the cat depended upon the stimulatory parameters. Stimulation of the dorsal hippocampus at lower frequencies resulted in the inhibition of ACTH release after painful stimuli. Stimulation at higher frequencies resulted in an increase of corticosteroid output.

Mason (1958) found marked elevation of plasma 17-OHCS levels after stimulation of the infundibular portion of the hypothalamus in monkeys. This change was not found after stimulation of the putamen or the anterior thalamus. Stimulation of the amygdaloid nucleus produced maximal increase in pituitary-adrenocortical activity. Hippocampus-fornix system exerts an unusual, prolonged suppressive action on the same system. The suppressor area may be responsible for the normal diurnal rhythm in ACTH secretion. Mason also postulated a cyclical mechanism from the reticular formation and hypothalamus up to the limbic system and back again, where the hippocampus is reciprocally concerned to the above areas. The hippocampus acts as a negative feed back on the hypothalamus-reticular formation when they receive stimulation through the ascending fibres.

Knigge (1961) said that lesions of hippocampus in rats increased basal plasma free corticosterone levels. Lesions in amygdala depressed the rate of adrenocortical response to the stress of immobilisation. The reaction was slow to develop and reached a maximum of  $37.0 \pm 3.8$  microgram/100 ml. at 4 hours. Fendler *et al.* (1961) observed increased adrenocortical secretion after hippocampal lesions.

Yamada and Greer (1960) found that bilateral electrolytic destruction of the amygdala in male rats did not significantly alter the thyrotropin or corticotropin secretion by the pituitary, but gonadotropin production was definitely reduced. The lesioned rats were adipsic and aphagic. As the thymic involution was noted in all the lesioned rats, the adrenal hypertrophy might be apparently related to increased adrenocortical secretion.

Mason (1961) observed a prolonged depression of the basal plasma corticosteroid level in monkeys after electrical stimulation of the Ammon's horn.



Ahrén (1962) found partial inhibition of both acute and chronic responses after lesions of the rostral limbic structures (anterior preoptic area and orbitofrontal cortex) and also in the anterior hypothalamic area.

Okinaka *et al.* (1960) studied the effect of electrical stimulation of the limbic system on pituitary-adrenocortical function (posterior orbital surface). They stimulated the posterior orbital surface of the morphine pre-treated dog electrically for 10 seconds under direct vision after craniotomy. Sharp and transient rise of both ACTH and 17-OHCS was noted immediately after the stimulation. These changes were not found after stimulation of the anterior sylvian gyrus, in hypophysectomized ACTH maintained animals, and in sham operated animals. Bilateral splanchnicectomy did not prevent the rise of blood ACTH in response to posterior orbital surface stimulation, but the onset of increase was considerably delayed.

The amygdala is not only responsible for respiratory changes (Kaada, 1951; Liberson *et al.*, 1951; Gastaut, 1952; MacLean and Delgado, 1953; Kaada *et al.*, 1954; Poirier and Shulman, 1954; Anand and Dua, 1955 and 1956) and cardiovascular changes (MacLean, 1952; Gastaut, 1952; Koikegami *et al.*, 1953; MacLean and Delgado, 1953; Poirier and Shulman, 1954; Chapman *et al.*, 1954; Baldwin *et al.*, 1954; Anand and Dua, 1955 and 1956) on stimulation but certain endocrine responses are also obtained after stimulation or lesion of this area of the brain. The adrenocortical responses have been mentioned before.

### Cingulate Region and Adrenocortical Response

Behavioural changes were noted in monkeys by Smith (1945) and Ward (1948) during experimentation on the anterior cingulate area. Smith noted greater tameness and Ward found loss of fear. Glees and others (1950) confirmed such observations in monkeys but these changes are not permanent after lesions and they disappear after six weeks to three months. It is found that apprehensiveness and anxiety disappear after ablations of the cingulate and so there is a place of neurosurgery in agitated, aggressive and over-active psychotic subjects and during anxiety and obsessional state (Barris and Schuman, 1953; Kennard, 1955; Le Beau, 1952; 1954; Le Beau and Petrie, 1953; Livingston, 1953; Scoville, 1954; Tow and Armstrong, 1954; Tow and Whitty, 1953; Ward, 1948; Whitty, 1955; and Whitty *et al.*, 1952).

Kennard (1955) noted confused, perseverative and obsessive behaviour, plasticity of posture and slight increase in rage reactions when bilateral ablation of the cingulate was done in the cat.

Smith (1945), Kremer (1947) and Ward (1948) found increased pilo-erection and salivation after ablation of the cingulate. Ward (1948) and Glees *et al.* (1950) noticed increased motor activity after bilateral lesion of the anterior cingulate in monkeys. Fulton (1951) said that "since it now seems clear that the cingulate is an important supressor region acting through the caudate nucleus, this observation is clearly of significance."

Anterior cingulate has also important function, possibly in connection with vocalization and speech (Smith, 1945; and Kaada *et al.*, 1949).



Fulton (1951) further states, "it is our impression, furthermore, that bilaterally cingulectomized monkeys are less prone to vocalize than normal monkeys, and that when they do respond to the call of other animals, the note emitted is much more of a guttural sound than the high-pitched chirp by which normal monkeys seem to communicate or at least to express their emotions."

Bilateral ablation of the cingulate region in monkeys produced a quiet, passive and soft animal. It lost aggressive tendencies. There was no hyperactivity and no perseveration. There was also some return to more normal function, and increase in the variability of performance (Kennard, 1955/6). Kennard also performed some combination ablations including the frontal poles, the temporal poles and the anterior cingulate region in monkeys. The cingulate and the mesial portion of the temporal pole are to be considered as one functionally undifferentiated unit. Functionally these areas are directly related to the frontal and temporal associative areas.

Connections of the cingulate region have been well described by Crosby *et al.* (1962).

Increased plasma 17-OHCS level in lightly anaesthetised cats was noted by Setekleiv *et al.* (1960, 1961) after stimulating the anterior cingulate cortex, the lower portion of the posterior ecto and supra-sylvian gyri of the temporo-occipital cortex and the amygdala.

Bohus (1961) studied the effect of central nervous lesions on pituitary-adrenocortical function in the rat. Bilateral electrocoagulation of the rat's anterior cingular area was made. This procedure significantly increased adrenocortical cortico-steroid output when compared with the secretion found in sham-operated rats, in rats with unilateral cingular lesion or bilateral occipital cortical lesion, as well as with that of normal control animals.

Ibayashi *et al.* (1963) stimulated the anterior and posterior parts of the cingular gyrus electrically in dogs. A quick rise in ACTH concentration in the jugular venous blood occurred. This rise was accompanied by changes in autonomic function manifested as pupillary dilatation, respiratory arrest, blood pressure changes and urination.

### Septal Region and ACTH Secretion

Heath (1954) paid much importance to the septal region of the brain and he defined the area as follows :—The septal region of the brain is formed posteriorly by the anterior commissure and the rostral area by the tip of the anterior horn of the lateral ventricle. Medially it extends to the midline of the brain, dorsally it extends to the septum pellucidum and to the base of the lateral ventricles, ventrally to the base of the brain and laterally about 5 mm. from the midline.



Developmentally the septal region is derived from the rhinencephalic part of the forebrain. Hypothalamus, just caudal to septal region is derived from the primitive diencephalon.

Its location is such that it is in between the higher neocortical level and the diencephalic and mid brain structures.

After ablation of this area in cats there were metabolic changes which are noted after adrenalectomy but the pituitaries and adrenals were normal or hypertrophied suggesting the primary nature of the neural mechanism.

Heath *et al.* (1954) stated that stimulation of the septal region in monkey produced increase of urinary 17-ketosteroid with marked decrease in the number of circulating eosinophils and lymphocytes, but these types of changes did not take place after stimulation of caudate nucleus and hippocampus. They also stated that stimulation of the septal region in man produced marked decrease in the circulating eosinophils and lymphocytes along with increase in the total white cell count. Stimulation of the caudate region failed to produce this change, whereas, there was often a certain degree of lymphocytosis and eosinophilia.

Pool *et al.* (1956) noted the steroid hormonal response to stimulation by electrodes implanted in the subfrontal parts of the human brain. They suggested the possibility of specific activating and perhaps depressing pathways for steroid production.

Bohus (1961) observed increased adrenocortical corticosteroid output in rats after lesion of the septal region. This increase was less than that induced by cingular lesion.

Endrőczy and Lissák (1960) studied the effect of electrocoagulation of the septal region on behaviour and endocrine system in 18 cats. According to the localisation of the lesion the results could be divided into two groups. (1) Animals with lesion in the sub and supracallosal regions surrounding the corpus callosum had marked elevation of corticoid content after 7 to 14 days of the operation. In these animals stimulation of the erogenic zone led to rage reaction without the copulative activity or lordosis in the female cats. (2) Other brain lesion reached the septum and some involved the sub-callosal area. The lesion was placed in the side of the lateral ventricle between the anterior commissure and the corpus callosum. There was destruction of the lateral part of the corpus callosum in some cases. In these animals either there was no change in the pituitary-adrenocortical activity or there was marked decrease in the corticoid content of the adrenal venous blood. In the later cases sexual or rage reaction could not be elicited by stimulation of the erogenic zone.

Katsuki (1961) observed adrenal atrophy after lesions of the ventro-caudal septum in rabbits.

Ahrén (1962) noted accentuated adrenocortical hyperplasia after lesions in the ventrocaudal septum in male rabbits of the same order as that due to lesions of the mammillary bodies. Septal lesions partially inhibited the acute response and the thymus atrophy. According to the author the ventrocaudal septum mediates both stimulatory and inhibitory influences.





### Mid brain and ACTH Secretion

Transection of the midbrain cuts off the peripheral nervous stimuli entering into the hypothalamus. After such lesion Davis *et al.* (1961) found elevation of corticosterone secretion rate after abdominal surgery in the dog. Martini *et al.* (1960) found blockage of the response in rat. Anderson *et al.* (1957) noted that chronic midbrain section in dogs abolished the adrenocortical response to different types of ACTH releasing stimuli. The adrenals were responsive to ACTH. Newman *et al.* (1958) observed that in cats transection of the midbrain reticular formation did not change adrenal secretion rate of aldosterone or cortisol. But destruction of the ventral diencephalon or specific lesions in the midbrain reticular formation diminished the aldosterone and cortisol secretion rate.

Szentagothai (1958) showed that the inhibiting effect of cortisone on ACTH secretion was absent in animals with electrolytic mesencephalic lesions. Martini *et al.* (1960) noted that unilateral adrenalectomy did not give rise to depletion of adrenal ascorbic acid in the remaining adrenal of animals with midbrain lesion. Moll (1959) did not find compensatory adrenal hypertrophy after unilateral adrenalectomy in animals with electrolytic mesencephalic lesions.

Endrőczy *et al.* (1961) found significant reduction in the corticosteroid content of the adrenal venous blood of rats and cats after injection of cortisone into the reticular formation. Davidson and Feldman (1963) observed a partial depression of compensatory adrenal hypertrophy in rats after double implants of cortisol into the reticular formation. Smelik and Sawyer (1962) could not block stress response after implantation of cortisol in midbrain of rabbits.

Mangili *et al.* (1964) noted high resting levels of blood corticosterone in midbrain sectioned animals.

Fraschini *et al.* (1964) put forward the hypothesis that in addition to an ACTH activating, an ACTH inhibiting and stress facilitating system, the midbrain might lead to ACTH control by acting as a type of modulator.

The experimental preparations in this part include the following :—

1. Control experiments for brain operations.
2. Anterior hypothalamic lesioned animals.
3. Animals with bilateral hippocampal lesion.
4. Bilateral amygdaloid lesion
5. Septal lesion.
6. Cingulate lesion.

### MATERIALS AND METHODS

Male dogs of 10 to 15 kg. in weight were used in this experiment. Intravenous nembutal anaesthesia was administered in a dose of 30 mgm./kg. of body weight. Right lumbo-adrenal vein was cannulated after the method of Hume and Nelson (1955). Burn trauma was produced by



immersing the extremity in boiling water (100°C) for 30 seconds under nembutal anaesthesia. Adrenal venous 17-OHCS has been estimated after the method of Silber and Porter (1954). Sterile techniques have been used all throughout the procedures except in the very acute experiments. Animals exhibiting intracranial infections were excluded from the series. In brain lesion-experiments the extent of lesion was determined by staining the sections with Nissl stain.

### I. *Control experiments for brain operations*

In this group 10 male dogs of 10 to 15 kg. in weight were used. Cannulation of lumbo-adrenal vein was done 48 hours before the control brain operation. This operation includes the exposure of the brain after incision over the meninges through the trephine hole. The layers were closed as usual without any brain lesion. Burn trauma to the right hind limb was inflicted at 60 minutes after this procedure in 5 dogs, and at 48 hours in 5 dogs. Adrenal venous blood 17-OHCS output was measured at 1/2, 1, 2, 3 and 6 hours after burn. Then intravenous ACTH (0.8 unit/kg.) was administered and the adrenocortical response was noted.

### II. *Anterior hypothalamic lesion*

Five male dogs of 11 to 15 kg. in weight were used. Lumbo-adrenal vein was cannulated 48 hours before the brain lesion. Anterior hypothalamic lesions were carried out in a dog through transbuccal approach by means of a bent tipped sucker. Standardized burn trauma was applied to the right hind limb 60 minutes after the preparation and adrenal venous corticosteroid output was measured at 1/2, 1, 2 and 3 hours after burn. ACTH response was noted.

### III. *Bilateral hippocampal lesion*

In this group 10 male dogs of 10 to 15 kg. in weight were used. Lumbo-adrenal vein was cannulated 48 hours before hippocampal lesion. Bilateral surgical lesion of the hippocampus was done in a single stage after the method of Bard and Mountcastle (1948). A segment of the fornix was also extirpated on each side. Rest for 48 hours was allowed to the animals. The corticosteroid output was measured. Standardized burn trauma was inflicted on the right hind limb and 17-OHCS output was measured at 1/2, 1, 3 and 6 hours after burn. ACTH response was then noted.

The blood pressure tracing during surgery is shown in fig. 1.

### IV. *Bilateral amygdaloid lesion*

Five male dogs of 10 to 14 kg. in weight were used. Right lumbo-adrenal vein was cannulated 48 hours before the brain lesion was produced. Lesion on both sides was produced in a single stage after the method of Bard and Mountcastle (1948). Burn trauma was produced on the right hind limb 48 hours after the brain lesion. Corticosteroid output was



measured at 1/2, 1, 3 and 6 hours after burn. ACTH response was subsequently noted.

Fig. 2 shows the blood pressure response during surgery.

#### V. *Bilateral septal lesion*

Five male dogs of 10 to 14 kg. in weight were used. Right lumbo-adrenal vein was cannulated 48 hours before the brain lesion. Bilateral septal lesion was carried out through the lateral ventricles in one stage after the method of Heath *et al.* (1954). Right hind limb was burned 48 hours after the brain lesion and adrenal venous 17-OHCS output was measured at 1/2, 1, 3 and 6 hours after burn. ACTH response was noted.

#### VI. *Bilateral cingulate lesion*

Six male dogs of 10 to 14 kg. in weight were used. Lumbo-adrenal vein cannulation was done 48 hours before the brain lesion was produced. Bilateral lesion of the cingulate was produced in one stage by the following steps :—

1. Parasagittal trephining of the bones and enlargement of the openings in an antero-posterior direction were carried out on both sides.
2. Dura was incised and reflected.
3. Exposed cerebral veins entering into the sagittal sinus were cauterized.
4. Gentle traction over the hemisphere exposed the cingulate area which was removed by suction. The same procedure was applied to the other side.

Right hind limb was burned 48 hours after the brain lesion. Adrenal venous 17-OHCS output was measured at 1/2, 1, 3 and 6 hours after burn. ACTH response was also noted.

### RESULTS

#### I. *Control experiments for brain operations*

In this group adrenal venous 17-OHCS output was high during cannulation operation in comparison to the post-cannulation values (48 hours after cannulation).

Subgroup—*a* (Table 1 and statistical table no. 1 : fig. 3)—During the surgery of control brain operations the corticosteroid output was significantly high at 30 and 60 minutes. Thirty minutes after burn the corticosteroid output was significantly high (0.1% level) when compared to the value at 60 minutes after brain operation. At 1, 2, 3 and 6 hours after burn the difference was significant at 1% level. Intravenous administration of ACTH showed good adrenocortical response six hours after burn.



Subgroup—*b* (Table 2 and statistical table no. 2 ; fig. 4)—After the control brain operations the high 17-OHCS output gradually reached a low level at 48 hours. The corticosteroid output was high upto 6 hours after burn and the adrenal cortex was well responsive to intravenous ACTH.

## II. *Anterior hypothalamic lesion* (Table 3 and statistical table 3 and 8 ; fig. 5).

The basal corticosteroid output (48 hours after cannulation) was significantly high at 60 minutes after the hypothalamic lesion. Thirty minutes after burn trauma it was significantly high (at 5% level). The output was further elevated at 60 and 120 minutes after burn. The peak level of the output was at 120 minutes. Intravenous ACTH administration led to further rise in corticosteroid output.

When the results of this group were compared to those of the control brain operation-group, significant differences were observed at 60 minutes after the preparations and 30, 60 and 180 minutes after burn. At 120 minutes after burn there was no difference in the responses.

The magnitude of response after burn was more in dogs with brain removed upto thalamohypothalamic level at 30, 60 and 180 minutes. At 2 hours there was no difference. Regarding the response after ACTH the corticosteroid output was more in dogs with brain removed upto thalamohypothalamic level (Table 8 and statistical table 19).

## III. *Bilateral hippocampal lesion* (Table 4 and statistical table 4 ; fig. 6)

Thirty minutes after bilateral hippocampal lesion the corticosteroid output was significantly higher in comparison to the basal level obtained 48 hours after adrenal vein cannulation. This high level persisted even at 48 hours after the brain lesion. The corticosteroid output was significantly high at 30, 60, 180 and 360 minutes after burn. The adrenals were well responsive to intravenous ACTH.

Photographs of bilateral lesion of hippocampus have been presented in figures 7 (dog no 190), 8 (dog no. 194), 9 (dog no. 197) and 10 (dog no. 199).

*Comparison of the results of this group with those of the control brain operations* (statistical table 9).

Forty eight hours after control brain operations the corticosteroid output reached a basal level, but the level was high in the bilateral hippocampal lesioned dogs. After burn and ACTH the responses were more in the latter group.

## IV. *Bilateral amygdaloid lesion* (Table 5 and statistical table 5 and 10 ; fig. 11).

Animals with bilateral amygdaloid lesion had increased 17-OHCS output at 30 minutes and 48 hours after the brain lesion. Burn trauma manifested with high corticosteroid output. Intravenous ACTH had good response.





Forty eight hours after control brain operation the corticosteroid output was low, but at this period it was high in amygdaloid lesioned dogs. After burn the differences were noted only at 3 and 6 hours, the maximum response was with the amygdaloid lesioned dogs.

Photographs of bilateral lesion of amygdala have been presented in figures 12 (dog no. 200) and 13 (dog no. 202).

V. *Bilateral septal lesion* (Table 6 and statistical table 6 and 11 ; fig. 14).

With bilateral septal lesions the dogs had high 17-OHCS output at 30 minutes and 48 hours after the brain operation. With burn trauma and intravenous ACTH there was further increase in the corticosteroid output.

Forty eight hours after control brain operation the corticosteroid output was at a basal level, but it was significantly high at this period in the septal lesioned dogs. After burn the responses were more in the septal lesioned dogs than in dogs with control brain operations except at 60 minutes after burn. Response after intravenous ACTH was more in the septal lesioned dogs.

Figures 15 (dog no. 205) and 16 (dog no. 207) show lesion of the septal region.

VI. *Bilateral cingulate lesion* (Table 7 and statistical table 7 and 12 ; fig. 17).

After bilateral cingulate lesion the basal level of 17-OHCS output was high at 30 minutes and at 48 hours after the brain lesions. Such dogs had increased 17-OHCS output after burn trauma at 30, 60, 180 and 360 minutes. ACTH response was significant at 5% level.

Dogs with bilateral cingulate lesion had high 17-OHCS output at 48 hours after the brain operation, whereas at this hour, dogs with control brain operations had low output. Responses after burn trauma and ACTH were maximum in the cingulectomized dogs.

Photographs of lesion of the cingulate region have been presented in figures 18 (dog no. 210) and 19 (dog no. 214).

*Intergroup comparisons of the adrenocortical responses in different types of brain lesioned dogs*

*Hippocampal lesioned dogs : Septal lesioned dogs* (Statistical table 16)

There was no difference in the comparisons except at 60 minutes after burn where the difference was significant at 1% level and the adrenocortical response was more in hippocampal lesioned dogs.

*Septal lesioned dogs : Amygdaloid lesioned dogs* (Statistical table 17)

Significant difference in the adrenocortical response was observed only at 180 minutes after burn and the maximum response noted was in Septal lesioned dogs.



*Hippocampal lesioned dogs : Amygdaloid lesioned dogs* (Statistical table 13)

Significant difference in the adrenocortical response was observed at 30 minutes after the brain lesions. Maximum response was noted in the hippocampal lesioned dogs at 30 and 60 minutes after burn.

*Septal lesioned dogs : Cingulate lesioned dogs* (Statistical table 18)

Forty eight hours after brain lesions maximum adrenocortical response was noted in the septal lesioned dogs. Three hours after burn and after intravenous ACTH administration the responses were more in the septal lesioned dogs.

*Hippocampal lesioned dogs : Cingulate lesioned dogs* (Statistical table 14)

The adrenocortical responses were more in the hippocampal lesioned dogs at 30 and 60 minutes after burn.

*Amygdaloid lesioned dogs : Cingulate lesioned dogs* (Statistical table 15)

There was no significant difference in the adrenocortical responses between the two groups.

*Tables overleaf*



TABLE I  
Control experiments for the brain lesioned dogs and burn (Fig. 3)

Dog numbers Observation numbers	137	138	139	140	141	Mean	S.D.	D.F.
	Adrenal venous 17-OHCS output (micrograms/minute)							
1. During adrenal vein cannulation	8.2	7.8	9.5	10.1	11.0	9.320	1.326	4
2. Forty-eight hours after cannulation	1.9	2.6	3.1	2.0	3.5	2.620	0.691	4
3. Thirty minutes post-operative	9.5	10.2	11.6	12.0	12.5	11.160	1.262	4
4. Sixty minutes post-operative	9.0	7.6	11.2	10.5	10.8	9.820	1.494	4
5. Thirty minutes after burn	20.1	25.0	23.7	25.0	20.5	22.860	2.401	4
6. Sixty minutes after burn	18.7	21.3	22.0	21.3	17.0	20.060	2.124	4
7. Two hours after burn	17.2	22.3	20.0	18.5	16.4	18.880	2.349	4
8. Three hours after burn	17.6	19.6	16.7	19.7	15.0	17.720	1.994	4
9. Six hours after burn	15.3	18.5	17.4	16.6	15.6	16.680	1.314	4
10. ACTH (I.V.)	21.0	24.8	21.5	24.0	22.7	22.800	1.611	4



TABLE II

*Control experiments for the brain lesioned dogs and burn (Fig. 4)*

Dog numbers Observation numbers	152	153	154	155	156	Mean	S.D.	D.F.
							Adrenal venous 17-OHCS output (micrograms/minute) %	
1. During adrenal vein cannulation	..	10.7	8.6	7.9	10.9	8.9	9.400	1.330
2. Forty-eight hours after cannulation	..	2.8	1.7	1.6	2.1	2.0	2.040	0.472
3. Thirty minutes post-operative	..	12.5	12.0	10.5	11.5	10.2	11.340	0.976
4. Sixty minutes post-operative	..	7.4	9.3	8.6	11.0	8.5	8.960	1.328
5. Three hours post-operative	..	8.0	8.6	7.6	9.2	7.5	8.180	0.716
6. Six hours post-operative	..	7.8	8.0	8.0	7.3	7.2	7.660	0.385
7. Twenty-four hours post-operative	..	4.1	5.5	3.0	4.5	3.6	4.140	0.945
8. Forty-eight hours post-operative	..	2.4	3.0	2.1	2.8	2.5	2.560	0.351
9. Thirty minutes after burn	..	24.6	21.6	18.4	22.6	19.9	21.420	2.396
10. Sixty minutes after burn	..	21.7	20.3	17.5	21.0	16.6	19.420	2.242
11. Two hours after burn	..	17.4	18.5	16.4	18.9	17.9	17.820	0.978
12. Three hours after burn	..	18.5	16.0	14.3	17.0	15.5	16.260	1.585
13. Six hours after burn	..	13.0	12.8	11.7	14.6	12.8	12.980	1.040
14. ACTH (I.V.)	..	21.0	20.3	20.0	23.4	22.4	21.420	1.443



TABLE III  
*Lesion of the anterior hypothalamus and burn (Fig. 5)*

Dog numbers Observation numbers	185	186	187	188	189	Mean	S.D.	D.F.
	Adrenal venous 17-OHCS output (micrograms/minute)							
1. During adrenal vein cannulation	..	10.8	7.0	7.4	8.5	9.2	8.580	1.517
2. Forty-eight hours after cannulation	..	3.0	2.3	1.8	2.5	2.9	2.500	0.485
3. Sixty minutes after the preparation	..	8.9	6.4	8.5	7.4	7.5	7.740	0.986
4. Thirty minutes after burn	..	11.2	7.1	9.0	9.4	8.3	9.000	1.508
5. Sixty minutes after burn	..	13.5	10.1	13.0	12.6	12.5	12.340	1.306
6. Two hours after burn	..	18.4	19.5	17.7	18.6	13.9	17.620	2.177
7. Three hours after burn	..	12.0	12.1	9.6	10.2	8.9	10.560	1.436
8. ACTH (I.V.)	..	16.1	18.5	13.0	19.4	11.7	15.740	2.597



TABLE IV  
*Bilateral lesion of the hippocampus and burn (Fig. 6)*

Dog numbers Observation numbers	190	191	192	193	194	195	196
	Adrenal venous 17-OHCS output (micrograms/minute)						
1. During adrenal vein cannulation	8.1	9.5	7.0	6.0	11.5	8.0	9.0
2. Forty-eight hours after cannulation	1.6	1.8	1.6	1.5	2.3	2.1	1.8
3. Thirty minutes after the preparation	14.1	11.6	12.2	11.0	19.0	12.5	18.5
4. Forty-eight hours after the preparation	16.0	7.3	9.0	10.6	15.4	9.5	11.1
5. Thirty minutes after burn	30.7	28.8	30.2	29.5	31.6	27.6	32.0
6. Sixty minutes after burn	20.1	28.0	24.7	27.2	28.5	21.5	29.0
7. Three hours after burn	17.5	24.1	22.2	24.4	25.5	20.7	27.3
8. Six hours after burn	15.0	23.0	25.1	19.3	27.3	18.5	25.5
9. ACTH (I.V.)	20.5	30.1	28.5	26.0	30.2	22.1	30.3



(Contd. Table IV)  
*Bilateral lesion of the hippocampus and burn*

Dog numbers Observation numbers	197	198	199	Mean	S.D.	D.F.
Adrenal venous 17-OHCS output (micrograms/minute)						
1. During adrenal vein cannulation	7.1	10.6	7.5	8.430	1.715	9
2. Forty-eight hours after cannulation	1.6	3.1	2.4	1.980	0.503	9
3. Thirty minutes after the preparation	16.6	20.0	10.3	14.580	3.630	9
4. Forty-eight hours after the preparation	15.5	16.6	7.0	11.800	3.737	9
5. Thirty minutes after burn	25.0	30.7	27.4	29.350	2.175	9
6. Sixty minutes after burn	28.6	29.1	25.0	26.170	3.241	9
7. Three hours after burn	24.1	26.5	20.2	23.250	3.069	9
8. Six hours after burn	19.4	21.5	22.0	21.660	3.732	9
9. ACTH (I.V.)	24.0	23.0	26.1	26.080	3.615	9



TABLE V  
*Bilateral lesion of amygdala and burn (Fig. 11)*

Dog numbers Observation numbers	200	201	202	203	204	Mean	S.D.	D.F.
	Adrenal venous 17-OHCS output (micrograms/minute)							
1. During adrenal vein cannulation	..	8.0	9.4	6.0	8.1	7.8	1.216	
2. Forty-eight hours after cannulation	..	1.6	2.0	1.4	1.9	1.5	0.259	4
3. Thirty minutes after the preparation	..	11.2	12.5	10.1	9.6	8.5	1.532	4
4. Forty-eight hours after the preparation	..	13.8	9.1	11.2	8.0	9.0	2.316	4
5. Thirty minutes after burn	..	29.5	27.0	20.5	28.0	21.7	3.994	4
6. Sixty minutes after burn	..	25.0	20.5	18.4	20.1	15.3	3.529	4
7. Three hours after burn	..	21.0	18.5	22.0	24.2	15.7	3.278	4
8. Six hours after burn	..	17.5	20.3	20.0	25.0	13.6	4.175	4
9. ACTH (I.V.)	..	24.0	26.1	26.4	29.6	18.4	4.149	4



TABLE VI  
*Lesion of septal region and burn (Fig. 14)*

Dog numbers Observation numbers	205	206	207	208	209	Mean	S.D.	D.F.
	Adrenal venous 17-OHCS output (microgramma/minute)							
1. During adrenal vein cannulation	7.5	10.2	11.0	8.0	8.8	9.100	1.473	4
2. Forty-eight hours after cannulation	1.8	2.6	3.2	2.8	2.1	2.500	0.557	4
3. Thirty minutes after the preparation	11.3	14.7	12.1	10.5	12.4	12.200	1.581	4
4. Forty-eight hours after the preparation	14.3	12.0	15.4	12.3	11.6	13.120	1.645	4
5. Thirty minutes after burn ..	30.3	27.6	25.0	29.0	26.1	27.600	2.137	4
6. Sixty minutes after burn ..	22.7	18.6	19.1	21.0	22.0	20.680	1.785	4
7. Three hours after burn ..	27.6	23.4	26.3	22.7	28.1	25.620	2.449	4
8. Six hours after burn ..	23.7	22.0	21.3	26.7	19.5	22.640	2.723	4
9. ACTH (I.V.) ..	27.3	28.4	24.2	29.3	26.5	27.140	1.958	4



TABLE VII  
*Lesion of cingulate region and burn (Fig. 17)*

Dog numbers Observation numbers	210	211	212	213	214	215	Mean	S.D.	D.F.
				Adrenal venous 17-OHCS output (microgramma/minute)					
1. During adrenal vein cannulation	..	7.8	8.0	10.6	8.7	6.6	7.967	1.603	5
2. Forty-eight hours after cannulation	..	2.0	3.1	1.9	2.2	1.8	2.083	0.549	5
3. Thirty minutes after the preparation	..	16.5	13.2	11.5	12.0	10.2	12.350	2.286	5
4. Forty-eight hours after the preparation	..	12.4	10.6	9.2	10.3	8.2	10.317	1.478	5
5. Thirty minutes after burn	..	28.5	25.9	27.6	23.2	21.8	25.217	2.589	5
6. Sixty minutes after burn	..	25.3	21.7	25.2	20.4	23.0	23.000	1.946	5
7. Three hours after burn	..	23.6	18.5	22.3	19.2	21.4	20.617	2.122	5
8. Six hours after burn	..	26.0	23.5	21.2	22.3	20.7	22.300	2.179	5
9. ACTH (I.V.)	..	26.7	25.0	26.4	23.5	21.6	24.400	1.987	5



TABLE VIII  
*Brain removal down to thalamo-hypothalamic level (posterior connections remaining intact) and burn*

Dog numbers Observation numbers	167	168	169	170	171	Mean	S.D.	D.F.
	Adrenal venous 17-OHCS output (micrograms/minute)							
1. During adrenal vein cannulation	..	13.0	7.0	10.4	7.4	9.800	2.557	4
2. Forty-eight hours after cannulation	..	2.7	1.9	2.6	2.0	2.460	0.505	4
3. Thirty minutes after the preparation	..	1.5	6.4	7.0	5.0	5.400	2.336	4
4. Forty-eight hours after the preparation	..	1.2	6.0	10.5	6.4	7.120	4.106	4
5. Thirty minutes after burn	..	8.5	21.5	26.8	23.2	22.120	8.380	4
6. Sixty minutes after burn	..	13.4	17.8	20.2	18.0	18.880	4.218	4
7. Three hours after burn	..	12.7	19.0	14.5	14.1	16.140	3.355	4
8. Six hours after burn	..	13.0	22.9	18.2	21.3	18.300	3.622	4
9. ACTH (I.V.)	..	16.6	26.9	24.2	26.4	22.420	4.809	4



STATISTICAL TABLE 1

*Values of "t" together with d.f. for statistical tests of significance for different types of "intra-group" paired comparisons regarding adrenal venous 17-OHCS output in dogs (control experiments for the brain lesions).*

Observation No.	1 vs.	Observation No.	2	d.f.	"t"
				4	12.727***
"	2 vs.	"	3	4	-18.852***
"	2 vs.	"	4	4	-11.861***
"	4 vs.	"	5	4	-9.674***
"	4 vs.	"	6	4	-8.470**
"	4 vs.	"	7	4	-5.992**
"	4 vs.	"	8	4	-5.700**
"	4 vs.	"	9	4	-6.558**
"	9 vs.	"	10	4	-10.426***

STATISTICAL TABLE 2

*Values of "t" together with d.f. for statistical tests of significance for different types of "intra-group" paired comparisons regarding adrenal venous 17-OHCS output in dogs (control experiments for the brain lesions).*

Observation No.	1 vs.	Observation No.	2	4	16.652***
"	2 vs.	"	3	4	-26.050***
"	2 vs.	"	4	4	-9.816***
"	2 vs.	"	5	4	-16.373***
"	2 vs.	"	6	4	-18.647***
"	2 vs.	"	7	4	-4.497*
"	2 vs.	"	8	4	-1.905
"	8 vs.	"	9	4	-18.490***
"	8 vs.	"	10	4	-17.917***
"	8 vs.	"	11	4	-51.208***
"	8 vs.	"	12	4	-20.147***
"	8 vs.	"	13	4	-26.856***
"	13 vs.	"	14	4	-23.504***



STATISTICAL TABLE 3

Values of "t" together with d.f. for statistical tests of significance for different types of "intra-group" paired comparisons regarding adrenal venous 17-OHCS output in dogs (lesion of the anterior hypothalamus and burn).

Observation No.	1 vs.	Observation No.	2	D.F.	"t"
	1 vs.		2	4	11.992***
"	2 vs.	"	3	4	-11.173***
"	3 vs.	"	4	4	-3.405*
"	3 vs.	"	5	4	-17.761***
"	3 vs.	"	6	4	-8.869***
"	3 vs.	"	7	4	-3.452*
"	7 vs.	"	8	4	-4.405*

STATISTICAL TABLE 4

Values of "t" together with d.f. for statistical tests of significance for different types of "intra-group" paired comparisons regarding adrenal venous 17-OHCS output in dogs (bilateral lesion of the hippocampus and burn).

Observation No.	1 vs.	Observation No.	2	D.F.	"t"
	1 vs.		2	9	14.145***
"	2 vs.	"	3	9	-11.602***
"	2 vs.	"	4	9	-8.495***
"	4 vs.	"	5	9	-14.222***
"	4 vs.	"	6	9	-9.883***
"	4 vs.	"	7	9	-8.231***
"	4 vs.	"	8	9	-5.362***
"	8 vs.	"	9	9	-8.170***

STATISTICAL TABLE 5

Values of "t" together with d.f. for statistical tests of significance for different types of "intra-group" paired comparisons regarding adrenal venous 17-OHCS output in dogs (bilateral lesion of amygdala and burn).

Observation No.	1 vs.	Observation No.	2	D.F.	"t"
	1 vs.		2	4	13.733***
"	2 vs.	"	3	4	-13.810***
"	2 vs.	"	4	4	-7.778***
"	4 vs.	"	5	4	-8.991***
"	4 vs.	"	6	4	-8.047**
"	4 vs.	"	7	4	-5.900**
"	4 vs.	"	8	4	-3.756*
"	8 vs.	"	9	4	-14.228***



STATISTICAL TABLE 6

*Values of 't' together with d.f. for statistical tests of significance for different types of "intra-group" paired comparisons regarding adrenal venous 17-OHCS output in dogs (lesion of septal region and burn).*

Observation No.	1 vs.	Observation No.	2	D.F.	"t"
				4	12.916***
"	2 vs.	"	3	4	-13.197***
"	2 vs.	"	4	4	-15.000***
"	4 vs.	"	5	4	-11.393***
"	4 vs.	"	6	4	-6.643**
"	4 vs.	"	7	4	-11.312***
"	4 vs.	"	8	4	-6.752**
"	8 vs.	"	9	4	-7.131**

STATISTICAL TABLE 7

*Values of 't' together with d.f. of statistical tests for significance for different types of "intra-group" paired comparisons regarding adrenal venous 17-OHCS output in dogs (lesion of cingulate region and burn).*

Observation No.	1 vs.	Observation No.	2	D.F.	"t"
				5	9.265***
"	2 vs.	"	3	5	-11.667***
"	2 vs.	"	4	5	-15.361***
"	4 vs.	"	5	5	-17.107***
"	4 vs.	"	6	5	-13.407***
"	4 vs.	"	7	5	-9.875***
"	4 vs.	"	8	5	-18.047***
"	8 vs.	"	9	5	-2.954*

\* = Significant at 5% level

\*\* = Significant at 1% level

\*\*\* = Significant at 0.1% level or more stringent level



STATISTICAL TABLE 8

Showing d.f. and values of 't' s' and their significance for different types of "intergroup" comparisons.

Table 1 vs. Table 3		D.F.		"t"
Observation No.		Observation No.		
4	vs.	3	8	2.598*
5	vs.	4	8	10.931***
6	vs.	5	8	6.911***
7	vs.	6	8	0.880
8	vs.	7	8	6.515***

STATISTICAL TABLE 9

Showing d.f. and values of 't' s' and their significance for different types of "intergroup" comparisons.

Table 2 vs. Table 4		D.F.		"t"
Observation No.		Observation No.		
3	vs.	3	13	— 1.927
8	vs.	4	13	— 5.416***
9	vs.	5	13	— 6.447***
10	vs.	6	13	— 4.151**
12	vs.	7	13	— 4.726***
13	vs.	8	13	— 5.017***
14	vs.	9	13	— 2.733*

STATISTICAL TABLE 10

Showing d.f. and values of 't' s' and their significance for different types of "intergroup" comparisons.

Table 2 vs. Table 5		D.F.		"t"
Observation No.		Observation No.		
3	vs.	3	8	1.182
8	vs.	4	8	— 7.316***
9	vs.	5	8	— 1.882
10	vs.	6	8	— 0.235
12	vs.	7	8	— 2.468*
13	vs.	8	8	— 3.274*
14	vs.	9	8	— 1.867



STATISTICAL TABLE 11

Showing d.f. and values of 't' s' and their significance for different types of "intergroup" comparisons.

Table 2 vs. Table 6				D.F.	"t"		
Observation	No.	3 vs.	Observation	No.	3	8	— 0.103
..	8	vs.	..	4	8	8	—14.037***
..	9	vs.	..	5	8	8	— 4.304**
..	10	vs.	..	6	8	8	— 0.983
..	12	vs.	..	7	8	8	— 7.172***
..	13	vs.	..	8	8	8	— 7.408***
..	14	vs.	..	9	8	8	— 5.257***

STATISTICAL TABLE 12

Showing d.f. and values of 't' s' and their significance for different types of "intergroup" comparisons.

Table 2 vs. Table 7				D.F.	"t"
Observation No.	3 vs.	Observation No.	3	9	— 0.914
"	8 vs.	"	4	9	—11.373***
"	9 vs.	"	5	9	— 2.503*
"	10 vs.	"	6	9	— 2.839*
"	12 vs.	"	7	9	— 3.782**
"	13 vs.	"	8	9	— 8.718***
"	14 vs.	"	9	9	— 2.788*

STATISTICAL TABLE 13

Showing d.f. and values of 't' s' and their significance for different types of "intergroup" comparisons.

Table 4 vs. Table 5				D.F.	"t"
Observation No.	3 vs.	Observation No.	3	13	2.443*
..	4 vs.	..	4	13	0.857
..	5 vs.	..	5	13	2.559*
..	6 vs.	..	6	13	3.458**
..	7 vs.	..	7	13	1.730
..	8 vs.	..	8	13	1.122
..	9 vs.	..	9	13	0.569



STATISTICAL TABLE 14

Showing d.f. and values of 't' s' and their significance for different types of "intergroup" comparisons

Table 4 vs. Table 7			D.F.		"t"
Observation No.	3 vs.	Observation No.	3	14	1.343
"	4 vs.	"	4	14	0.920
"	5 vs.	"	5	14	3.433**
"	6 vs.	"	6	14	2.156*
"	7 vs.	"	7	14	1.839
"	8 vs.	"	8	14	-0.380
"	9 vs.	"	9	14	1.039

STATISTICAL TABLE 15

Showing d.f. and values of 't' s' and their significance for different types of "intergroup" comparisons

Table 5 vs. Table 7				D.F.	"t"		
Observation	No.	3 vs.	Observation	No.	3	9	—1.638
"	4	vs.	"	4	4	9	—0.984
"	5	vs.	"	5	5	9	0.062
"	6	vs.	"	6	6	9	—1.876
"	7	vs.	"	7	7	9	—0.206
"	8	vs.	"	8	8	9	—1.548
"	9	vs.	"	9	9	9	0.263

STATISTICAL TABLE 16

Showing d.f. and values of 't' s' and their significance for different types of "intergroup" comparisons

Table 6 vs. Table 4			D.F.		"t"
Observation No.	3 vs.	Observation No.	3	13	-1.381
"	4 vs.	"	4	13	0.744
"	5 vs.	"	5	13	-1.477
"	6 vs.	"	6	13	-3.490**
"	7 vs.	"	7	13	1.496
"	8 vs.	"	8	13	0.611
"	9 vs.	"	9	13	0.605



STATISTICAL TABLE 17

*Showing d.f. and values of 't' s' and their significance for different types of "intergroup" comparisons*

		Table 6 vs. Table 5		D.F.		"t"
Observation	No.	3 vs.	Observation No.	3	8	1.848
"	"	4 vs.	"	4	8	2.283
"	"	5 vs.	"	5	8	1.115
"	"	6 vs.	"	6	8	0.464
"	"	7 vs.	"	7	8	2.918*
"	"	8 vs.	"	8	8	1.507
"	"	9 vs.	"	9	8	1.092

STATISTICAL TABLE 18

*Showing d.f. and values of 't' s' and their significance for different types of "intergroup" comparisons*

		Table 6 vs. Table 7		D.F.		"t"
Observation	No.	3 vs.	Observation No.	3	9	-0.124
"	"	4 vs.	"	4	9	2.976*
"	"	5 vs.	"	5	9	1.641
"	"	6 vs.	"	6	9	-2.042
"	"	7 vs.	"	7	9	3.636**
"	"	8 vs.	"	8	9	0.231
"	"	9 vs.	"	9	9	2.293*



STATISTICAL TABLE 19

Values of 't' together with d.f. for statistical tests of significance for different types of "intra-group" paired comparisons regarding adrenal venous 17-OHCS output in dogs (brain removal down to thalamo-hypothalamic level—posterior connections remaining intact and burn)

Observation No.		D.F.	"t"
1 vs.	2	4	7.565**
2 vs.	3	4	— 2.753
2 vs.	4	4	— 2.630
4 vs.	5	4	— 7.440**
4 vs.	6	4	—19.216***
4 vs.	7	4	— 5.767**
4 vs.	8	4	— 5.526**
8 vs.	9	4	— 4.741**

STATISTICAL TABLE 20

Showing d.f. and values of 't' s' and their significance for different types of "intergroup" comparisons

Table 3 vs. Table 8	D.F.	"t"
3 vs.	8	0.328
4 vs.	8	—3.445*
5 vs.	8	—3.310*
6 vs.	8	0.828
7 vs.	8	—4.441**
8 vs.	8	—2.733*

\* = Significant at 5% level

\*\* = Significant at 1% level

\*\*\* = Significant at 0.1% level or more stringent level.



## DISCUSSION

*Anterior hypothalamic lesion and burn* :—Electrical stimulation and lesion of the hypothalamus have been performed by various authors in order to understand its role in hypophyseal ACTH release. The stimulation experiments have been performed by de Groot and Harris (1950), Hume and Wittenstein (1950), Porter (1953 and 1954), Anand and Dua (1955) and others. Lesion experiments were performed by de Groot and Harris (1950), Hume and Wittenstein (1950), McCann (1953), Porter (1953 and 1954), Anand *et al.* (1954), Keller *et al.* (1954), Laquer *et al.* (1955) and others. The precise location in the hypothalamus which when stimulated or lesioned, leads to increased ACTH release or blocks ACTH response to stress, is not identical in all the experiments. Therefore Akert (1959) has said that "these areas cannot be identified as *corticotrophic* any more than the afferent pathways by which stress signals are sent to the hypothalamus. The experiments merely confirmed the notion that the hypothalamus organizes patterns of defense over a wide range of efferent systems, one of them being the pituitary-adrenal system." Brodish (1963) has also observed that a diffuse hypothalamic nucleus or network controls ACTH secretion. The centre is not localised and discrete.

In the present investigation the corticosteroid output is significantly high at 60 minutes after anterior hypothalamic lesion. Such animals show increased corticosteroid output after burns and the maximum response is noted at 120 minutes, though the increased response starts at 30 minutes after burns. Dogs with control brain operation show maximum response after burn trauma. Adrenocortical response to burn trauma is more in dogs having the hypothalamus intact (brain removed upto thalamohypothalamic level) than in dogs with anterior hypothalamic lesion. The late maximum response in dogs with anterior hypothalamic lesion may be due to the action of burn toxins and corticotropin releasing factors (from sources other than the anterior hypothalamus) on the anterior pituitary, circulating via the systemic circulation. The liberation of CRF is the result of interaction between the stimulatory and the depressive areas of the brain after the stress impulse travels through the reticular formation of the brain.

Hume and Jackson (1959) noted intact adrenocortical response to trauma from 4 hours to 7 days after hypothalamic destruction in dogs. In the second week there was loss of normal response to trauma. Keller *et al.* (1954) observed usual eosinopenic response to surgery after ventral hypothalamectomy. Wise *et al.* (1964) speak about an ACTH stimulating humoral agent liberated from traumatized tissues in dogs with removal of hypothalamus. Brodish (1960) found a late adrenocortical response after stress in rats with anterior, middle or posterior hypothalamic lesions.

*Hippocampus and burn* :—Removal of rhinencephalic structure leads to behavioural changes and it is now certain that the whole limbic system has a pronounced influence over behaviour. Recent experiments tend to



prove that this system has great influence over the hypothalamopituitary adrenal-axis. Klüver (1958) states that "the tempororhinencephalic system with its poikilo functions is poised between isofunctions of the cortex guaranteeing an approximate constancy of the *external* environment and the isofunctions of the diencephalon guaranteeing an approximate constancy of the *internal* environment." The emotional pathway as considered by Papez (1937) was from the anterior thalamus→cingulate gyrus→cingulum→hippocampus→fimbria→fornix→mammillary body. From the mammillary body it came back to the anterior thalamus. Green (1960) suggested some of the functions of the hippocampus and these are :—(a) it is concerned in emotion, (b) it is concerned in visceral activity, (c) it is concerned with memory mechanisms, (d) it is part of a general forebrain suppressor system, and (e) its activity in some way, may be the converse of that of the neocortex on the reticular activating system.

Green (1960) mentioned that electrolytic or surgical lesions (incomplete destruction) within the hippocampus produced different changes which were just akin to those after local stimulation in the conscious state. Thus the results which we get may be due to destruction or due to irritation. Green (1958) said that two types of hippocampal injuries were met with in their preparations. The first was due to direct electrolytic lesions within the hippocampus. The second was seen after lesions in the amygdala. Secondary necrosis of the hippocampus after primary lesion in the postero-medial part of the amygdala may be due to interruption of the anterior choroidal vessels.

Dogs with bilateral hippocampal lesions manifest with high 17-OHCS output at 48 hours after the brain lesion. At this period dogs with control brain operations show basal corticosteroid output. This proves a checking influence of this area of the brain on the pituitary-adrenocortical system. After burn trauma there is a good response. The checking influence of the hippocampus was also noted by Porter (1954), Endr czi *et al.* (1954), Mason (1958), Mason (1961), Endr czi and Liss k (1962), Roy (1962) and others.

From Kraft's (1955) translation of "Studies on the cerebral cortex by Ramon Y Cajal" we find that structures entering and ending in Ammon's horn and the fascia dentata are the sphenocornual pathway, the cingulum, the nerves of Lancisio and the band of fibres coming from the cells of the subiculum (subiculo-cornual pathway). The centrifugal path of Ammon's horn is fimbria, its prolongation to the trigonal pillars and the radiation of the tuber cinereum. He thinks that the fornix longus of Forel is not related to Ammon's horn. It starts from the gyrus fornicatus and also from the inducium.

Lorente De No (1934), Adey and Meyer (1952), Green and Arduini (1954), and others thought that the hippocampus has afferents from the septum and entorhinal cortex. There was abolition of activation of the hippocampus after septal lesion, but removal of the entorhinal cortical areas unaffected it (Green and Arduini, 1954). From the works of Green and Adey (1956) there is evidence of direct connection between the hippo-



campus and same sided amygdaloid nucleus. The efferent fibres from the hippocampus go to the medial mammillary body via the fornix.

Crosby *et al.* (1962) describe the fimbria-fornix complex. Near the splenium of the corpus callosum the main bulk of the hippocampus is absent and the fimbria bundles run in four directions.

(1) Some of the fibres may distribute to the medial olfactory area or some may reach the induseum griseum.

(2) Some other fibers of fimbria (fornix dorsalis) enter the septum pellucidum and others join the main fornix. Others pass to the cornu ammonis and the entorhinal area of the opposite side.

(3) Septo-hippocampal fibers of the fimbria are afferents to the hippocampus.

(4) Other fibers of fimbria continue as fornix.

The main bulk of the fornix fibers courses along the lower border of the septum pellucidum with which it is connected by ingoing and outgoing fibers. At the anterior end the precommisural portion is connected with the septal nuclei. Fine fibers also reach the preoptic and the anterior hypothalamic areas. The post commissural fornix contains also septo-mammillary fibers and hippocampomammillary fibers. After stimulation of the hippocampus, marked projection to the mammillary body, anterior thalamus, mammillothalamic tract and the midbrain tegmentum has been noted. Some fibers of the fornix join septo-habenular path.

From the above mentioned description it is evident that the hippocampus is connected with different subcortical areas and through these connections it has got a checking influence over the pituitary-adrenocortical system. Green (1956) has said that "The hippocampus must perform some correlative function in receiving and distributing the nervous impulses which pass to it via the extralemniscal pathways. Adaptation is known to occur here and while a direct path from the septum to amygdala may well occur, modification in response to monotonous stimuli or stimuli with emotional content could well take place through this critical system."

*Amygdala and burn* :—When single shock stimuli are applied to the hippocampus, short latency-responses have been observed in the amygdala, specially in the areas of the anterior, central and basolateral nuclei by Green and Adey (1955). Amygdala is reciprocally interconnected with the pyriform cortex (Crosby and Humphrey, 1944 ; Pribram and MacLean, 1953 ; and Gloor, 1955). The entorhinal area has got important afferents to the hippocampus (Cajal, 1911 ; Lorente De No, 1933 ; 1934 and Allen, 1948). The amygdala projects to the preoptic area, the anterior thalamus, the dorsomedial, ventromedial and arcuate nuclei of the hypothalamus (Fox, 1940, 1943 ; Adey and Meyer, 1952 ; Nauta, 1956 ; Gloor, 1955 and others). Gloor (1955) has shown that amygdala activates through multisynaptic relays an area extending from the septum through the hypothalamus and subthalamus back to the rostral mesencephalic tegmentum. This may be through the medial forebrain bundle.



The amygdaloid complex can be grouped into corticomedial and basolateral group of nuclei. The previous one is phylogenetically old and the latter one is phylogenetically young. Fibers from the corticomedial nuclear group enter into the stria terminalis and reach the preoptic parolfactory areas of the same side and also of the opposite side. There are short connections between the two groups of the amygdaloid complex. Crosby *et al.* (1962) state that impulses from basolateral group are relayed through the hippocampal gyrus to the fornix system and thus the basolateral complex discharges into the hypothalamus and the mammillary body. The basolateral group also sends some fibres to the stria terminalis which reach the preoptic, anterior hypothalamic and also ventromedial hypothalamic areas. They further state that impulses from the basolateral and corticomedial group are relayed directly and indirectly to the hypothalamus. The basolateral group has indirect connection with the tegmentum of the midbrain through hippocampal gyrus. It is connected with the orbital surface of the brain by the uncinate fasciculus and with cingulate gyrus by the cingulum.

Gloor (1960) has discussed the olfactory afferents, other afferent sensory connections, and non-sensory afferent connection to amygdala.

From the present investigation we find that bilateral amygdaloid lesions lead to increase in 17-OHCS output 48 hours after the brain lesion. There is further elevation in the corticosteroid output after burn trauma. A dog with bilateral hippocampal lesion shows more corticosteroid output in response to burn trauma than that noted in a dog with bilateral amygdaloid lesion and burn. From the review we find that there are differences of opinion regarding the influence of amygdaloid complex on the pituitary adrenocortical axis. From the lesion experiments in this study it can be stated that the amygdala influences the axis by inhibitory impulses. Our surgical ablation did not allow us to restrict the lesion in a complexwise manner (corticomedial or basolateral complex). Moreover undesired hippocampal lesions are an associated finding through vascular jeopardisation. Thus the results that we have achieved may be due to an associated hippocampal lesion. The increased adrenocortical activity may also be due to the loss of hippocampal inhibition which is mediated through the great bulk of afferents of the amygdala from the hippocampus. The amygdaloid complex influences the hypothalamo-pituitary adrenal-axis through the pathways described above.

*Septal area and burn* :—Cajal (from Kraft, 1955) thought that physiologically, the septum should be taken as a nerve centre associated with or subordinate to Ammon's horn and the fascia dentata. Adey and Meyer (1952) and Green and Arduini (1954) found connections between septum and hippocampus. The latter authors found that septal lesions diminished the activation of the hippocampus. There are septo-hypothalamic connections and the hippocampal inter-connection is through the fornix system.

In the present investigation the septal lesion in dogs leads to enhanced pituitary adrenocortical activity and burn trauma increases the 17-OHCS output further. This is due to the destruction of the fibers of inhibitory



influence over the pituitary-adrenocortical system. Mason *et al.* (1957) found that stimulation of the septum in the monkey lowered the corticoid level in peripheral blood. Endrőczy and Lissák (1960) demonstrated that electrocoagulation of the septal area in the cat was followed by a rise in corticosteroid secretion. Bohus (1961) noted the same type of change in the rat.

*Cingulate area and burn* :—Cajal (from Kraft, 1955) thought that the whole of the supracallosal convolution did not correspond to the inter-hemispheric region in the cat or dog. The lower half or three-fourth of the gyrus supracallosus is analogous to the cingulate cortex of rodents. In rodents the cingulum arises from the whole of the internal surface of the hemispheres. The posterior prolongation of the cingulum subserves as one of the afferent pathways of Ammon's horn. The important afferent connection of the cingulate gyrus is from the anterior nuclear group of the dorsal thalamus. In the rabbit the supracallosal part of the cingulate area gets fibers from the anteroventral nucleus. The anterior nuclear group of the dorsal thalamus receives the mammillo-thalamic fibers. The efferent fibers from the cingulate gyrus are, cortico-striatal, cortico-thalamic, cortico-hypothalamic and cortico-tegmental.

Changes in visceral responses have been observed by many investigators after stimulation of the cingulate area or lesion of the same region (Spencer, 1894 ; Bailey and Sweet, 1940 ; Smith, 1944, and 1945 ; Kremer, 1947 ; Kaada *et al.*, 1949 ; Babkin and Kite, 1950 ; Kaada, 1951 ; Hess *et al.*, 1951 ; Anand and Dua, 1956 ; Showers and Crosby, 1958). Crosby *et al.*, (1962) suggest that "in normal behaviour, impulses from the cingulate gyrus tend to suppress undue hypothalamic discharges (including those related to emotional expression) and to balance with the hippocampal areas which are presumably activators of such discharges."

After bilateral lesion of the cingulate area in dogs we have observed rise in 17-OHCS output. Burn trauma led to further increase in the corticosteroid output. Therefore, the cingulate area has also got control over the activity of the hypothalamo-pituitary-adrenal-axis. Regarding its role on this axis we can say that it has got a balancing action between the hippocampus and the hypothalamus. Emotional pathway of Papez (1937) can also explain the control of the different areas of brain on the hypothalamo-pituitary-adrenocortical system.

*Central neural adaptation* :—The central nervous system not only controls the behavioural and emotional reactions but also takes part in the regulation of the activity of the pituitary-adrenal-axis. The adaptation activity of the body occurs through the intermediation of two systems, one humoral *i.e.* the peripheral endocrinal activity and the other the central nervous system acting through the pituitary. Between the central nervous system and the hypophysis there is the neurohumoral path, neural for the neurohypophysis and humoral for the adenohypophysis. There is a balance between the stimulatory and the depressive areas of the brain regarding the activity of the pituitary-adrenal-axis. During trauma (burn) this balance is upset and the stimulatory action predominates and adaptation ensues. In very critical situation which we encounter in our absolute



brain-less dogs none of these stimulatory or depressive areas are present. Now the absolutely solitary axis is confronted possibly with the interglandular adaptation and added stress to the animal is manifested with increased adrenocortical response. Thus, we understand that in an intact animal, adaptation to stress is sum-total of the endocrinological response and activity of the central nervous system which is also influenced humorally apart from being influenced by nerves. Adaptation in the solitary axis may also occur through humoral path. Hypophysectomized animals cannot cope with the stress situations properly and adaptation is imperfect. Selye (1936a, 1936b, 1937, 1946) showed in the rat that there was no enlargement of the adrenals and discharge of lipid granules from the adrenal cortex in hypophysectomized rats after trauma, toxic doses of drugs, emotional stress or acute starvation. Harkins (1944), Harkins and Long (1945), and Long (1947) noted fall in total adrenal cholesterol in rats after skin burns, but this response was not observed after hypophysectomy. Hypophysectomy prevents a sharp fall in the adrenal ascorbic acid and total cholesterol concentration in rats after severe haemorrhage. Long (1947) observed that hypophysectomy could prevent such changes in the adrenals after cold, painful stimuli and trauma.

Miller and Riddle (1942) noted however, that in the pigeon hypophysectomy did not prevent the enlargement of the adrenal cortex with other histological changes indicating hyperactivity after injection of formaldehyde.

### CONCLUSION

1. After lesion of the anterior hypothalamus the maximum adrenocortical response following burn appears late.
2. Adrenocortical response after burn trauma is more in dogs having the hypothalamus intact (brain removed upto thalamohypothalamic level) than in dogs with anterior hypothalamic lesion.
3. The hippocampus, the septum, and the cingulate area have got inhibitory influences over the pituitary adrenocortical secretion. After lesion of any of these areas there is increased adrenocortical activity and burn trauma increases the response further. The cingulate area has got a balancing action between the hippocampus and the hypothalamus regarding the pituitary-adrenocortical activity.
4. Amygdala seems to have a restraining influence over the pituitary-adrenocortical activity which may be due to the associated vascular lesion of the hippocampus. Burn trauma increases 17-OHCS output further in such dogs.

The different areas of the brain and the pathways responsible for the control of the pituitary-adrenocortical function are shown in fig. 20.

5. Adaptation activity also occurs at the brain level through different stimulatory and depressive areas of the brain and their afferent and efferent fiber system. The net result is a steady state in the body in adverse situations what Claude Bernard has said long time back. Thus apart from the endocrinal adaptation, the central neural integrations are also to be considered.





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## LEGENDS TO FIGURES

- Fig. 1. Blood pressure changes during bilateral hippocampal lesion.
- Fig. 2. Blood pressure changes during bilateral amygdaloid lesion.
- Fig. 3. Adrenal venous 17-OHCS output in control experiments for the brain lesioned dogs and burn.
- Fig. 4. Adrenal venous 17-OHCS output in control experiments for the brain lesioned dogs and burn.
- Fig. 5. Adrenal venous 17-OHCS output in lesion of the anterior hypothalamus and burn.
- Fig. 6. Adrenal venous 17-OHCS output in bilateral lesion of the hippocampus and burn.
- Fig. 7. Section through R15 (Lim's atlas) showing bilateral lesion of the hippocampus (dog No. 190).
- Fig. 8. Section through R10 (Lim's atlas) showing bilateral hippocampal lesion in dog No. 194.
- Fig. 9. Section through R15 (Lim's atlas) showing bilateral hippocampal lesion in dog No. 197.
- Fig. 10. Section through R15 (Lim's atlas) showing bilateral hippocampal lesion in dog No. 199.
- Fig. 11. Adrenal venous 17-OHCS output in bilateral lesion of amygdala and burn.
- Fig. 12. Section through R19 (Lim's atlas) showing bilateral amygdaloid lesion in dog No. 200.
- Fig. 13. Bilateral amygdaloid lesion in dog No. 202.
- Fig. 14. Adrenal venous 17-OHCS output in lesion of septal region and burn.
- Fig. 15. Section through R25 (Lim's atlas) showing lesion of septal region in dog No. 205.
- Fig. 16. Section through R25 (Lim's atlas) showing lesion through septal region in dog No. 207.
- Fig. 17. Adrenal venous 17-OHCS output in lesion of cingulate region and burn.
- Fig. 18. The two halves of the brain are put in retracted position to show the lesions in cingulate region in dog No. 210.
- Fig. 19. Section through R30 (Lim's atlas) showing lesion of the cingulate region in dog No. 214.
- Fig. 20. Figure showing brain areas controlling ACTH secretion.



Fig. 1—Blood pressure changes during bilateral hippocampal lesion.



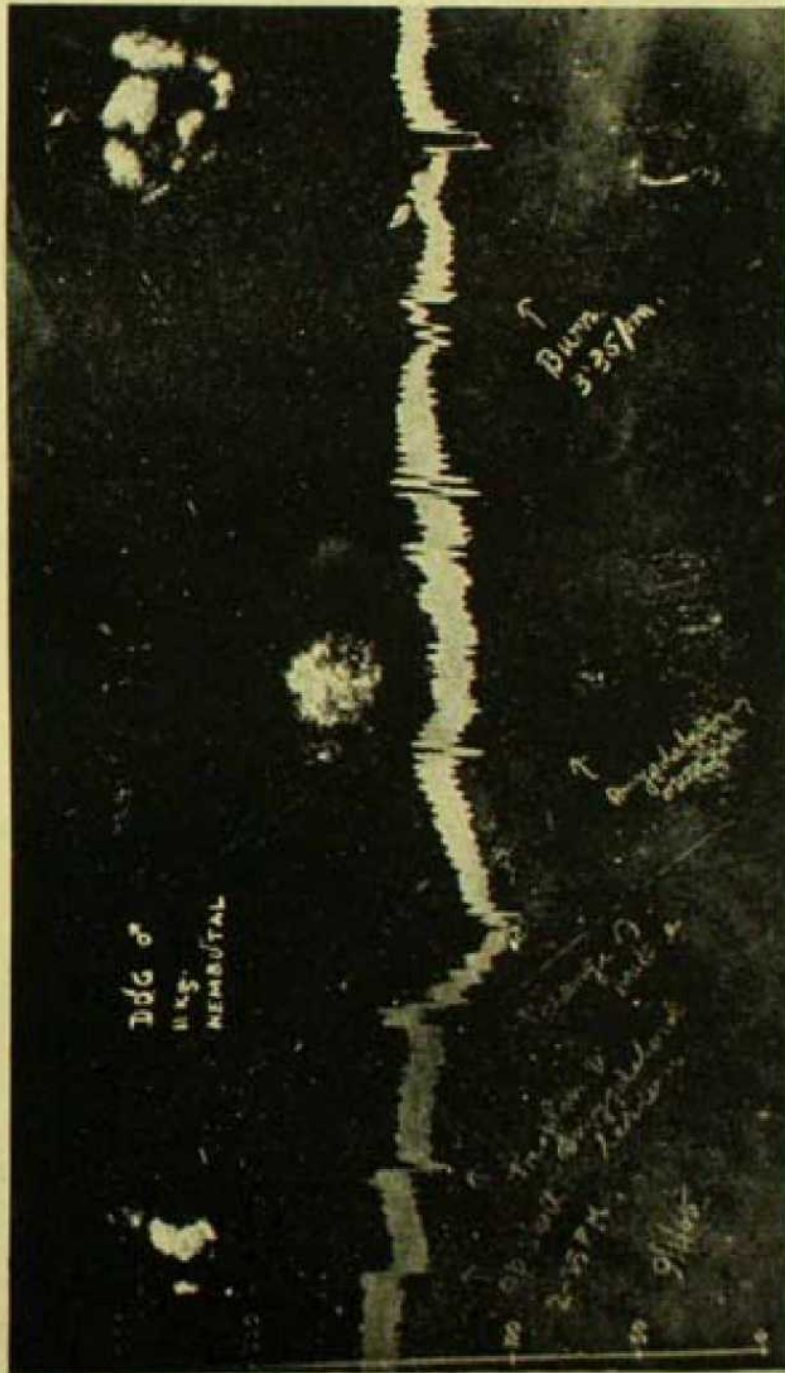


Fig. 2.—Blood-pressure changes during bilateral amygdaloid lesion.



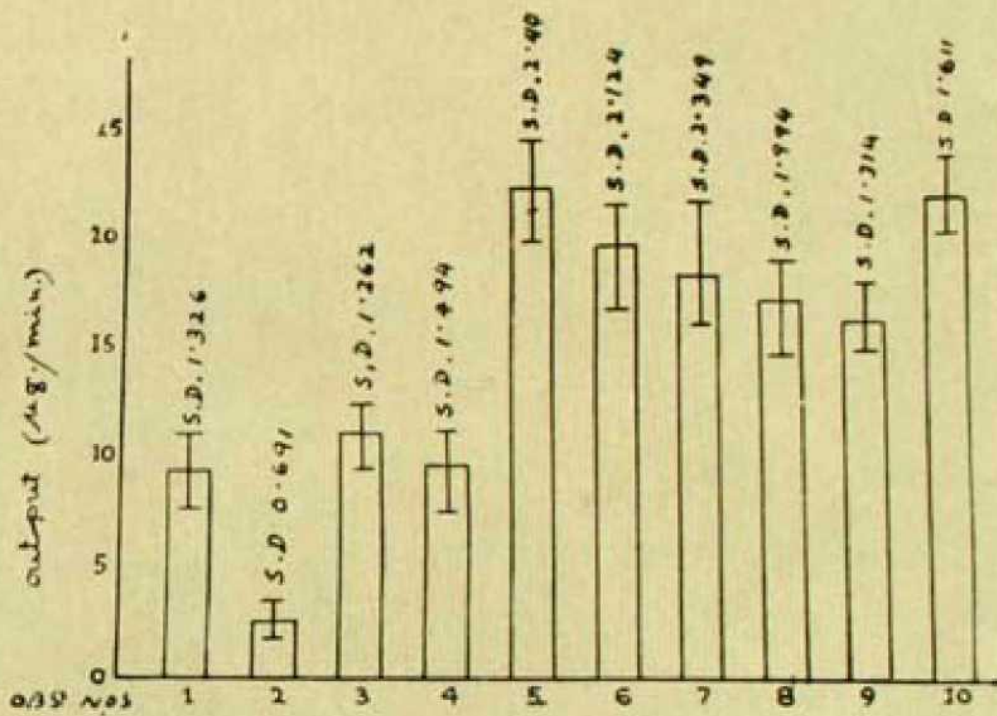


Fig. 3—Adrenal venous 17-OHCS output in control experiments for the brain lesioned dogs and burn.

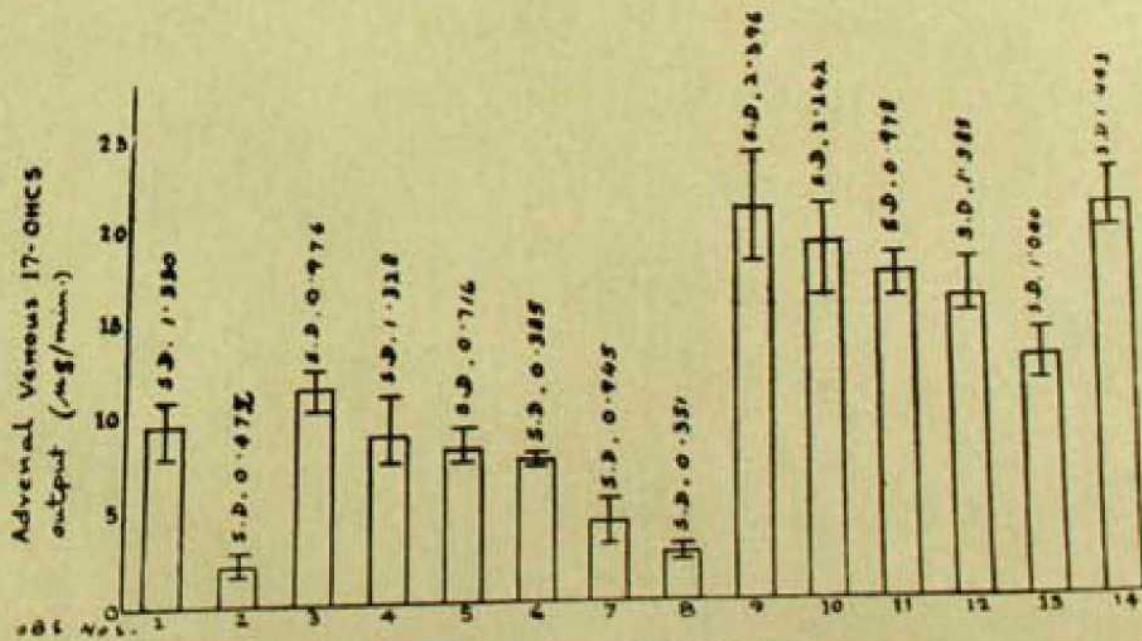


Fig. 4—Adrenal venous 17-OHCS output in control experiments for the brain lesioned dogs and burn.



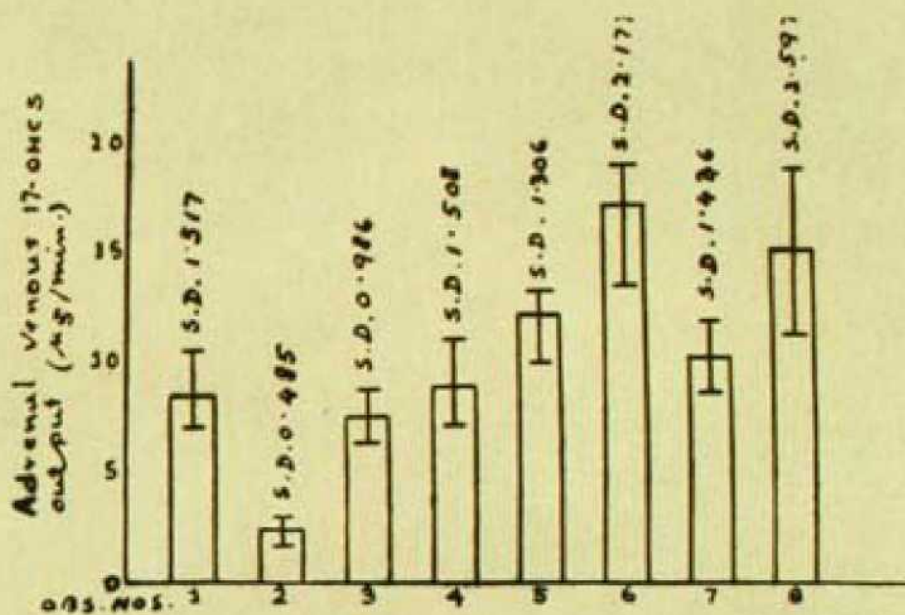


Fig. 5—Adrenal venous 17-OHCS output after lesion of the anterior hypothalamus and burn.

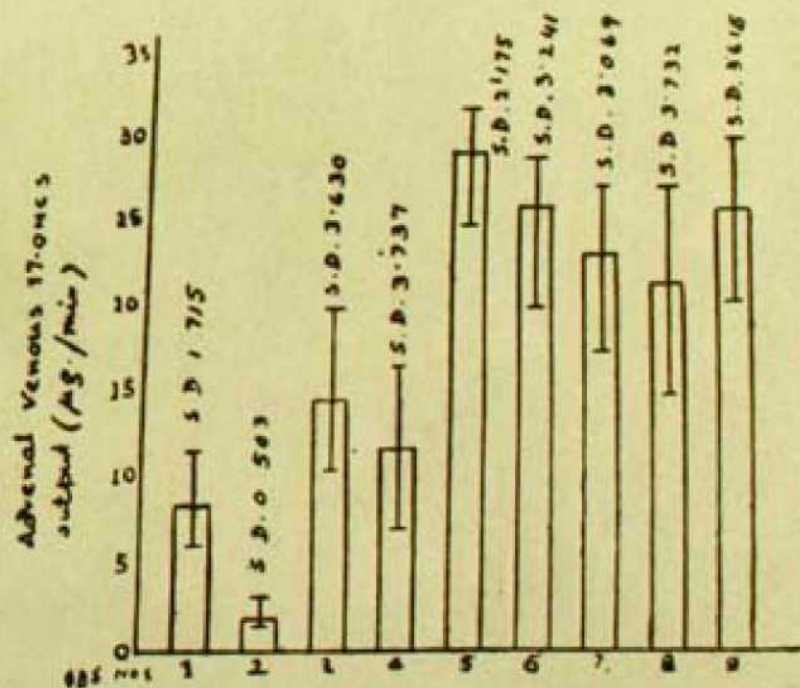


Fig. 6—Adrenal venous 17-OHCS output after bilateral lesion of the hippocampus and burn.





Fig. 7—Section through R 15 (Lam's atlas) showing bilateral lesion of the hippocampus (dog No. 190.)



Fig. 8—Section through R 10 (Lam's atlas) showing bilateral hippocampal lesion in dog No. 194.



Fig. 9—Section through R 15 (Lam's atlas) showing bilateral hippocampal lesion in dog No. 197.



Fig. 10—Section through R 15 (Lam's atlas) showing bilateral hippocampal lesion in dog No. 199.

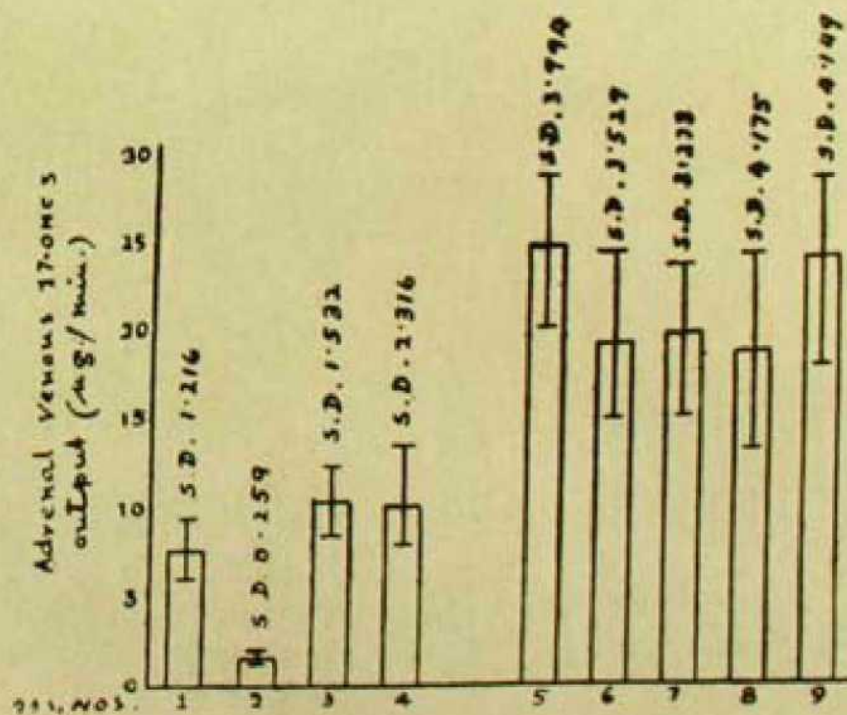


Fig. 11—Adrenal venous 17-OHCS output after bilateral lesion of amygdala and burn.



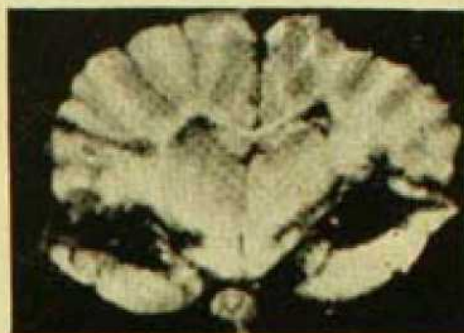


Fig. 12—Section through R 19 (Lim's atlas) showing bilateral amygdaloid lesion in dog No. 200.

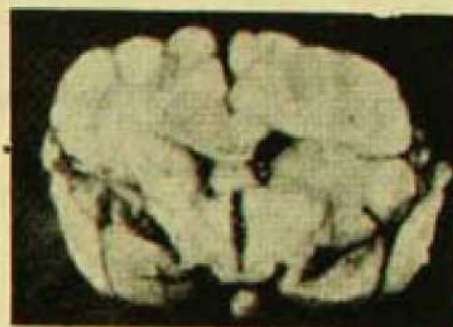


Fig. 13—Bilateral amygdaloid lesion in dog No. 202.

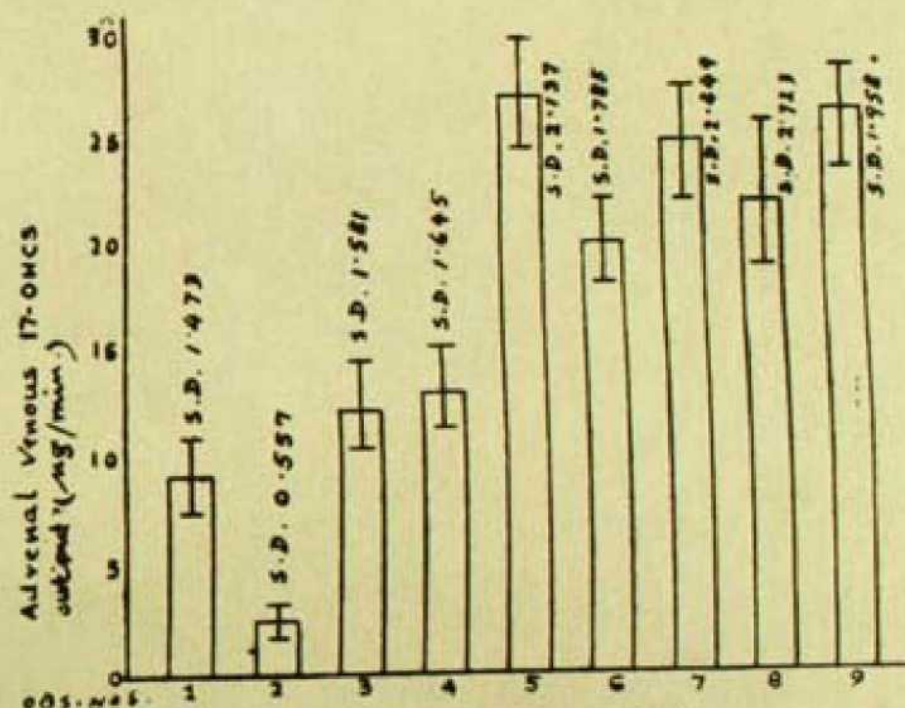


Fig. 14—Adrenal venous 17-OHCS output after lesion of septal region and burn.

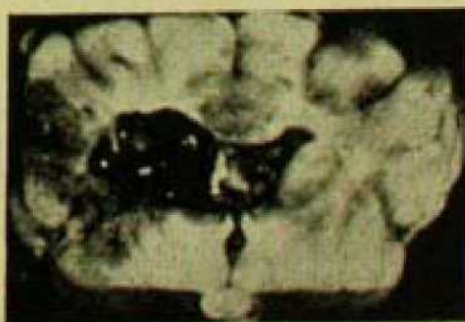


Fig. 15—Section through R 25 (Lim's atlas) showing lesion of septal region in dog No. 205.



Fig. 16—Section through R 25 (Lim's atlas) showing lesion through septal region in dog No. 207.



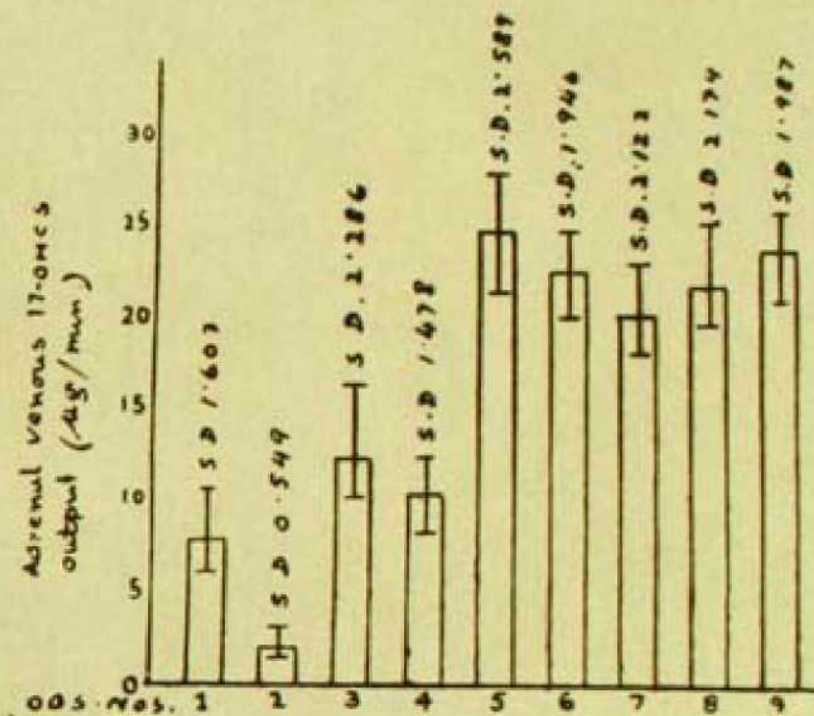


Fig. 17—Adrenal venous 17-OHCS output after lesion of cingulate region and burn.



Fig. 18—The two halves of the brain are put in retracted position to show the lesions in cingulate region in dog No. 210.



Fig. 19—Section through R 30 (Lam's atlas) showing lesion of the cingulate region in dog No. 214.



## BRAIN AREAS CONTROLLING A.C.T.H. SECRETION



## SECTION C

### BRAIN MECHANISMS RESPONSIBLE FOR ACTH RELEASE IN EXPERIMENTAL BURNS (1967)

#### ABSTRACT

Burn trauma is a continued type of stress with increased adrenocortical activity. In severely burned dogs patterns of increased adrenocortical activity in single or double extremity burn cannot be differentiated. ACTH cannot stimulate the maximally stimulated adrenal cortex further. Quick amputation of a burned extremity brings down the increased adrenocortical activity. Presence of *wound hormone* in the burned area is suspected because of the late rise in adrenocortical activity from the following experiments :—

- (a) Subtotal isolation of the hind limb with absence of the neural link.
- (b) Cross-circulation experiments—dog B is connected to the hind limb of dog A by only vascular pathway.
- (c) Limb grafting experiments.

That the presence of neural link is essential for prompt adrenocortical response after burn is proved by the following experiments :—

- (a) Single and double extremity burn groups.
- (b) Subtotal isolation of the hind limb with neurovascular link intact.
- (c) Cross circulation experiments—dog A whose isolated limb is connected to the rest of the body only by neural link.

Neurectomy after burn does not bring down the already increased 17-OHCS output immediately to a basal level. The fall depends upon time interval at which the neurectomy was done after burn.

Splanchnicectomy and partial sympathectomy have no effect on the quick increased response of the adrenals after burn. Prompt increased adrenocortical activity after ablation of central nervous system structures except the pituitary in splanchnicectomized and partially sympathectomized dogs after burn may be due to the action of the toxins on the isolated pituitary-adrenal-axis which becomes highly sensitive in such preparations.

#### INTRODUCTION

Regarding the pathways along which the stress message travels and also about the presence of any burn toxin, Hume and Egdahl (1959) and Egdahl (1959) conclude that in the burned area there is no humoral



stimulating substance and the adrenocortical response after severe burn is *solely* due to intact nerve supply from the burned area.

An attempt has been made here to explain some of the problems regarding the pituitary-adrenal-axis after experimental burn trauma. In the present work we have tried to find out whether the burned limb can produce some substance which can activate the pituitary-adrenal-axis in absence of neurogenic path or after ablation of central nervous system structures. Effect of amputation of burned extremity on the same axis has been noted. Importance of nervous influence towards the center and the effect of splanchnicectomy and partial sympathectomy on adrenocortical response after burn has been studied.

(a) *Role of Afferent Nerves in the Mediation of the Stress Response*

Gordon (1950) thought of the importance of the afferent nerves in adrenocortical response after trauma of lesser magnitude. The response was blocked after neurectomy. Severe scald however, led to adrenal ascorbic acid depletion even after neurectomy. Hume (1952) found no eosinopenia after operative injury to a somatically and sympathetically deafferented extremity of a dog. But there was slight eosinopenia after a very severe burn injury. Long (1950) did not get eosinopenia in spinal rats after laparotomy and intestinal manipulations at one hour but at four hours eosinopenia developed. Anderson (1954) failed to observe any stimulation of the pituitary-adrenal system after laparotomy and hemidecortication by measuring the urinary formaldehydogenic substance in dogs after cervical cord section. Wilson *et al.* (1956) observed adrenocortical stimulation after laparotomy in spinal dogs; but this response was blocked when the section was done at the level of the midbrain. Anderson *et al.* (1957) studied the effects of midbrain and spinal cord transection on endocrine and metabolic functions and they postulated a midbrain hypothalamico-pituitary activating system. Release of ACTH was disturbed after transection of the midbrain. There was no rise in ACTH secretion, and TSH secretion appeared to be depressed. Return of TSH secretion to normalcy came after about two weeks. In a dog which survived midbrain transection operation for more than one year, release of ACTH after stress was permanently blocked. There was no interference with ACTH release mechanism after transection of the spinal cord at the lower cervical level.

Hume and Wittenstein (1950) did not get eosinopenia in dogs with cord section after trauma to the extremity. Similar result was found by Hume (1953). Severe scald however, led to some eosinopenia, much less than normal.

Hume (1958) reported the results of cord section experiments on dogs and studies on human paraplegics and quadriplegics. In dogs, three days after cord section at C7, there was release of ACTH in response to laparotomy. The response started slower than in the intact animal. In human paraplegics and quadriplegics there was no rise in peripheral blood 17-hydroxycorticosteroid after operations involving skin, muscle and bone below the level of cord section, but the adrenals were responsive to exogenous ACTH. Operation above the level of cord section in one





patient led to an increase in peripheral corticosteroids. These results suggest that afferent nerve impulses are important for the release of ACTH after operative trauma, but in dog experiments some substance is released in the injured tissues which is transported through the blood stream to the brain. Thus peripheral neurovascular link is essential for the occurrence of stress induced ACTH release. These act at the level of anterior median eminence-postoptic area for release of some anterior pituitary-ACTH liberating substance as lesions of this area of the brain in dogs markedly depress secretion of ACTH after laparotomy. This suggests that nervous and humoral stimuli influence the pituitary through this area of the diencephalon. The difference in the results noted in dog and in man may be due to the different types of surgical stress in the two groups or it may be due to chronic lesions in man and acute (3 days after cord section) lesions in dogs.

Hume and Egdahl (1959) repeated the cord section experiments in dogs and they studied also isolated-leg dogs. They conclude that in the burned area there is no humoral stimulating substance. The adrenocortical response after a severe burn is *entirely* due to the intact nerve supply from the burned area. Pain factor is not also required as the response to burn is under nembutal anaesthesia and even when the brain cortex is removed. In their experiment number three and dog number W-25 (burn to denervated leg) however, there was a rise in adrenal venous corticosteroid at thirty minutes.

Egdahl (1959) could not find ACTH release after burn or surgical incision in denervated hind limbs of dogs. Redgate (1962) concluded that no contribution to ACTH release was rendered by the vascular path.

Kadas *et al.* (1959) studied the effects of spinal cord transection on the corticosterone secretion and histologic pattern of the adrenal cortex. Spinal cord section abolished the response to stress.

According to Moore (1957) some substance or substances from the wound itself initiated the diffuse change in metabolism. Severe tissue injury started post-traumatic metabolism when there was constant supply of pituitary and adrenal hormone. He said that in this sense the hormones were permissive and the initiative substance was a chemical or chemicals from the wound itself which may be called "wound hormone." If there was no rise in the hormone levels after severe injury there was question of survival and homeostasis was broken. But there was a rise by neuroendocrine mechanism and moreover, by reduced clearance from the blood.

Hume *et al.* (1962) found that spinal cord transection at the level of the 4th thoracic vertebra abolished ACTH release after laparotomy in man. However, Osborn *et al.* (1961) reported normal adreno-cortical responses to surgery below the level of cord section. These three patients had complete transections of the spinal cord at the level of C-6 to C-7.

Robinson and Munro (1958) reported impairment of adrenocortical function in patients having spinal cord transection above the 5th thoracic vertebra. However, patients with lesions below this level secreted normal adrenal steroid hormones.



Redgate (1960) found no release of ACTH in rats after application of electrical stimuli to the part below the level of cord section. Such stimuli applied to the part above the level of cord section manifested with increased ACTH secretion as measured by fall in adrenal ascorbic acid.

Eisenstein *et al.* (1962) studied adrenocortical hormone secretion in patients with spinal cord transection by measuring urinary and plasma 17-OHCS before and after ACTH and by noting any diurnal variation in plasma adrenal steroid levels. These values when compared to those for control subjects did not show any difference in hormone secretion. Steroid degradation in cord-sectioned patients showed no difference with that of the controls.

#### (b) *Burn Stress and Adreno-Cortical Secretion*

Rapid loss of adrenal cholesterol was noted in the rat after burn by Harkins (1944), Harkins and Long (1945), Long (1947), Ludewig and Chanutin (1947) and Sayers (1949). In guinea-pigs and rats there was more rapid loss of ascorbic acid (Miyagi, 1939; Long, 1947; Ludewig and Chanutin, 1947; Sayers, 1949).

Depression of eosinophil cell count after burn was noted by Evans and Butterfield (1951), Wight *et al.* (1953) and Hardy (1955). Sevitt (1951 and 1954) observed eosinopenia only for one or two days in patients with burns affecting less than 10% of the body surface and the eosinopenia lasted for 3 to 5 days in more severely burned subjects. In very ill patients the count was low for weeks but this was higher than that noted in the immediate post-burn period. Roy (1959) observed a close correlation of the burn with the fall of the eosinophil cell count in lesser degree of burns and the period of eosinopenia was short. The fall was complete or nearly complete in severe burn and the duration of eosinopenia was prolonged because of prolonged adrenocortical hyperactivity. Eosinopenia was noted during change of dressings, in wound infection, during and after skin grafting, after thrombophlebitis and lung complications and during other sorts of complications. Cases showing prolonged eosinopenia without rebound phenomenon were prognostically bad.

Sevitt (1957) discussed the problem of adrenocortical failure in burns. Acute adrenal failure might occur either at the adrenal level or at the hypothalamo-pituitary level. Feller (1962) also noted relative and differential adrenocortical insufficiency after burns.

Roy (1953) noted high levels of bio- and chemocorticoid contents of the adrenal venous effluent in the burned dogs. Hume *et al.* (1956) found increased level of 17-OHCS in plasma and its urinary excretion was high during the first two weeks in more extensively burned subjects. Increased urinary excretion of 17-ketosteroid was noted after burns by Cope *et al.* (1943), and Wardlaw (1950). Normal level of urinary 17-ketosteroid was noted by Hardy (1955) in relatively mild burn. Increased corticoid excretion was found in burned subjects, (Heard *et al.* 4, 1946, Tompsett and Oastler, 1947, Talbot *et al.*, 1947; Evans and Butterfield, 1951; Browne, 1951; Roy, 1953; Hardy, 1955; Hume *et al.*, (1956).

Timmer (1960) observed about 50% ACTH loss from the anterior pituitary within 12 hours after scald, Holub *et al.* (1959) observed almost



similar results. Knigge *et al.* (1959) observed a rise in pituitary ACTH content after similar condition. Rennels and Timmer (1962) noted increased plasma corticosterone level in rats after scalding.

### (c) *Influence of Splanchnic Nerves on Adrenocortical Secretion*

Vogt (1944) observed increased adrenocortical secretion in half of the experiments with dogs and cats after electrical stimulation of the left splanchnics, provided reflex activity of the nerves on both sides was avoided. Splanchnicectomy did not alter the *basal* output of the suprarenal cortex. Vogt (1947) noted that the denervation of the adrenals did not prevent the lipid depletion in rats after the following types of stress : (a) exposure to low temperature (+2 to +4°C) for 16 hours, (b) exposure to high temperature (39°C) for 2 to 6 hours (provided a rise in body temperature was produced, and (c) loss of blood amounting to 2% of the body weight. Okinaka *et al.* (1954) found increase of the chemo-corticoid concentration in the adrenal venous blood after stimulation of the splanchnic. This was noted even after hypophysectomy. Histologically there was adrenocortical hyperactivity. Hume and Nelson (1955) noted increased adrenocortical secretion after haemorrhagic shock in splanchnicectomised and partially sympathectomized dogs. This suggested that adrenal medulla did not participate in the increase of adrenocortical secretion in shock.

Okinaka *et al.* (1960) studied the effect of splanchnic nerve stimulation on adrenocortical secretion in dogs. This procedure increased the cortisol/corticosterone ratio in adrenal venous blood.

Lever (1953) found a fine and extensive nerve plexus in the adrenal cortex of the rat by using a special technique. Two varieties of these fibres were noted : (a) a fine plexus along with the cortical arteries possibly extending over capillary walls was found, and (b) the second type was of branching systems of fine and frequently beaded fibres. These had occasional buttonshaped endings in close proximity to the cortical cells. The two different types of fibres were derived from subcapsular plexus where darkly stained ganglion cells sometimes could be found. The above findings suggest direct adrenocortical nerve supply to the outer cell layers over and above a possible vasomotor innervation.

Regarding the innervation of the cortical cells different opinions exist ; some are in favour of it and others are against it (Stohr, 1935 ; Hoshi, 1926 ; Hollinshead, 1936 ; Kiss, 1951 ; Bennett, 1940 ; McFarland and Davenport, 1941 ; Bourne, 1961 and others).

### (d) *Burn Toxins*

In the leading article on toxæmia of burns in the Lancet (1960) it has been asked "are some deaths due to non-bacterial toxæmia ?" Robertson and Boyd (1923), Bernhard (1936) and Wilson *et al.* (1937) observed severe illness or death of normal animals after injection of extracts of burned skin or exudate. Parascandolo (1904), Pfeiffer (1905) and Pfeiffer (1913) noted similar results after injection of blood or serum from burned animals. Underhill and Kapsinov (1931), Harrison and Blalock (1932), Harkins *et al.* (1935) and Krauel and Payne (1958) could not find any serious effect.



Vogt (1912), Cevario (1921), Vaccaressa (1922) and Christophe (1939) did cross circulation experiments between normal and burned animals. The normal animals became ill or died. This may mean that a toxin from burned animal has reached the circulation of the normal one and has manifested the illness. Oligaemia itself can explain the situation also. Rydigier (1888), Salvioli (1891), Vaccaressa (1922) and Christophe (1939) found good results after exclusion or excision of the part.

Wilson (1946) said that the changes in the liver and kidney in burns without tannic acid treatment did not suggest the cause being shock only but also some added toxin was almost certainly present.

Sevitt (1957) listed the abnormal substances released or formed after burn. These are haemoglobin, pyronin-stainable substance and nuclear nucleoprotein, skin-proteinase, polypeptides, histamine, abnormal proteins, peptidase and others.

Feodorov (1956) suggested that autoimmunisation regularly occurred after burns and the antigens might produce the burn illness. Specific protein antigens were noted in the blood and tissues of the burned animals. Serum and blood from convalescent dogs after burns when transfused into recently burned dogs were much beneficial. There was increase of survival time and mortality rate diminished. He also noted the presence of immune sera in normal dogs when injection of blood from burned animals was given to them.

Mason *et al.* (1936) did not find any delay in the absorption and excretion of potassium iodide from the burnt area.

Schwalb (1959) studied the effect of isoindoline on survival of rats subjected to acute thermal burn. He noted that when the temperature of hot-water-burn was increased in rats from 85°C to 95°C, there was a significant decrease of mean survival time. Isoindoline (0.2 mg. per rat) given immediately after burning led to a significant increase of mean survival time in all temperature ranges tested. The effect of the blocking agent was most obvious at 90°C.

Van Caneghem (1951) and Simonart (1955) studied the toxicity of proteins heated above 80°C. Simonart (1958) and Godfraind (1958) have noted high toxicity in peptone oedema. Allgower and Siegrist (1957) and Allgower, Aschieri and Hulliger (1961) have injected heated proteins, specially blood constituents intraperitoneally. Allgower (1962) said that he could not rule out absolutely the damaging effect of burn tissue. He says that there are two difficulties in the experimental analysis of hypothetical burn toxins. (1) It is extremely difficult to differentiate the effect of pure tissue toxin from that of bacterial infection and intoxication. (2) If heated and sterilised tissues are put into healthy animals, inflammatory reaction and loss of fluid complicate the picture. He concludes that time and temperature factors are required for the damaging effect of the burned tissue.

Rosenthal (1959) discussed about the "burn toxin" and he said that products of burn diffusate definitely enter the circulation of burned rats. The higher the antigenic content of the diffusate, the greater the toxicity. The



diffusates were obtained directly from the burned skin of rats circumventing the circulation and these were lethal to mice and rats when injected subcutaneously, intravenously, intraperitoneally or intracerebrally. The toxic factor/s is dialyzable, heat stable and can be only partially precipitated by 80% ethanol. The toxic material contains peptides, polynucleotides, hexoses and pentoses. Rosenthal further stated that histamine, bradykinin, adeny compounds might be contributory factors but are not the "burn toxins."

## MATERIALS AND METHODS

Male dogs of 10 kg. to 15 kg. in weight were used in these experiments. Intravenous nembutal anaesthesia was administered in a dose of 30 mgm./kg. of body weight. Right lumbo-adrenal vein was cannulated after the method of Hume and Nelson (1955). Burn trauma was produced by immersing the extremity in boiling water (100°C) for 30 seconds under nembutal anaesthesia (except in group VIII). All surgical measures were carried out aseptically under nembutal. 0.8 units of ACTH/kg. was used intravenously or locally as mentioned in the tables. The experimental set up has been divided into the following groups.

### *Group—I :—Burn Injury Applied to Single Hind Limb (right side)*

In this group 15 male dogs of 10 to 15 kg. in weight were used. The lumbo-adrenal vein was cannulated forty eight hours before the burn experiment. Pre-and post-burn blood samples were collected from the cannula at different intervals and 17-OHCS was estimated after the method of Silber and Porter (1954).

### *Group—II :—Burn Injury Applied to Both Hind Limbs*

Five male dogs of 10.5 to 15 kg. in weight were used. Subsequent methods are same as in group I.

### *Group—III :—Amputation of a Hind Limb*

Fifteen male dogs of 10 to 15 kg. in weight were used. The lumbo-adrenal vein was cannulated forty eight hours before the amputation. Under nembutal anaesthesia amputation of the right hind limb was done through the hip joint. The bleeding points were secured and ligated with catgut and the wound was closed in layers as usual. A corrugated rubber drain was put into the wound and dressings were applied.

### *Group—IV :—Burn and Amputation*

In this group, amputation of the burned hind extremity has been done at different intervals (one hour, two hours and three hours) after burn injury in order to understand the following :—

- (a) the time of normalisation of adrenocortical secretion.
- (b) whether amputation at different hours after burn injury has any influence over the adreno-cortical secretion.

Sixteen adrenal vein-cannulated male dogs (10 to 15 kg. in weight) have been selected for this study. The standardized burn injury was



applied to the right hind limb and amputation through the hip joint was performed in 5 dogs after one hour, 6 dogs after two hours and 5 dogs after three hours. Adrenal venous blood samples were collected at intervals.

*Group—V :—*

In this group the principle of the study is to note the role of neurovascular influence on corticoid secretion after burn injury applied to a hind limb by the following procedures.

A. Subtotal isolation of the extremity keeping only the femoral artery, femoral vein and the sciatic nerve intact (five male dogs of 10 to 14 kg. in weight) (Fig. No. 1).

B. Subtotal isolation of the extremity keeping only the femoral artery and femoral vein, the nerve being divided during the isolation procedure (Eight male dogs of 10 to 14 kg. in weight) (Fig. No. 2).

Experimental subdivision is required as we want to avoid reburning of the same burned extremity. Chronic preparation is not used as this leads to—(a) huge oedema of the deserted extremity, (b) absorption from an oedematous tissue is much hindered and (c) nerve regeneration is expected until otherwise obstructed and thus may vitiate the evaluation of the results.

The right lumbo-adrenal vein was cannulated after the method of Hume and Nelson (1955) for intermittent collection of adrenal venous blood. Forty-eight hours after cannulation, a mid-thigh amputation (right side) is carried out under intravenous nembutal anaesthesia leaving either femoral artery, vein and sciatic nerve intact or femoral artery and femoral vein only sectioning the sciatic nerve. Bleeding points are secured and ligated. Perivascular sympathectomy is carried out at the same time. This procedure not only helps in damping the sympathetic afferentation but also ensures good circulation to the deserted limb. Standardized burn is inflicted to the extremity. 17-OHCS output is measured after the method of Silber and Porter (1954).

In some experiments with the nerve and vessels intact, the nerve is sacrificed after burn at different periods to note the influence of post-burn neurectomy on corticoid output.

C. Subtotal isolation of the right hind limb was done keeping the neurovascular link intact. Neurectomy was performed 30 minutes after burn (Six male dogs of 10.5 to 15 kg. in weight).

D. Subtotal isolation of the right hind limb was done keeping the neurovascular link intact. Neurectomy was performed 60 minutes after burn (Six male dogs of 10 to 15 kg. in weight).

E. Subtotal isolation of the right hind limb was done keeping the neurovascular link intact. Neurectomy was performed 180 minutes after burn (Six male dogs of 10.5 to 15 kg. in weight).

*Group—VI :—Cross-Circulation Experiment (Fig. No. 3)*

The left hind limb of dog A receives vascular supply from neck vessels of a second animal *i.e.* dog B. The hind limb of dog A is totally separated



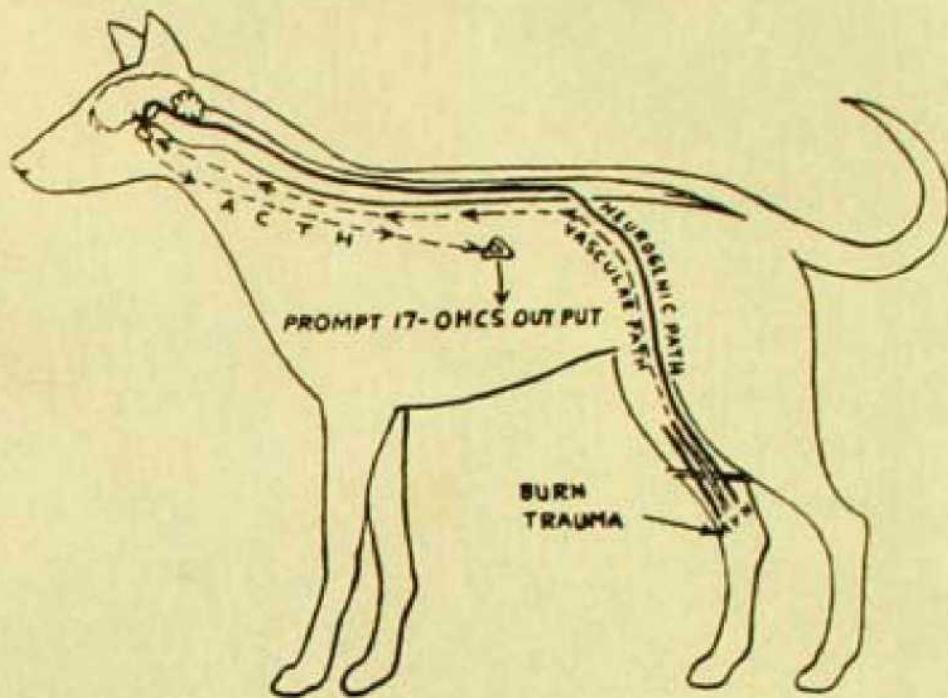


Fig. 1—Subtotal isolation of hind limb with neurovascular link intact,

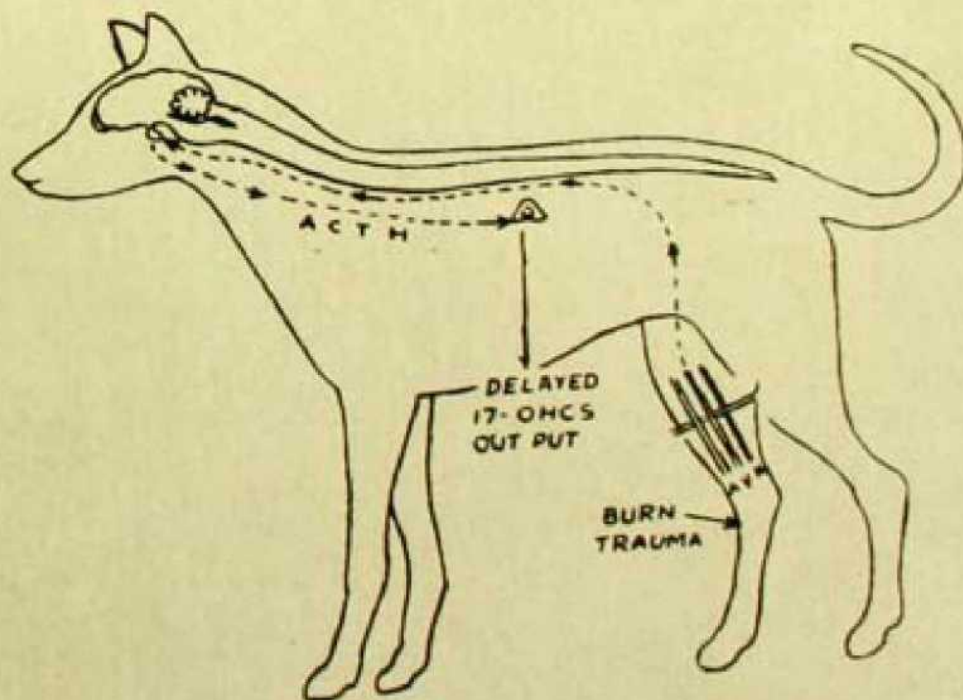


Fig. 2—Subtotal isolation of the hind limb with only vascular link intact,



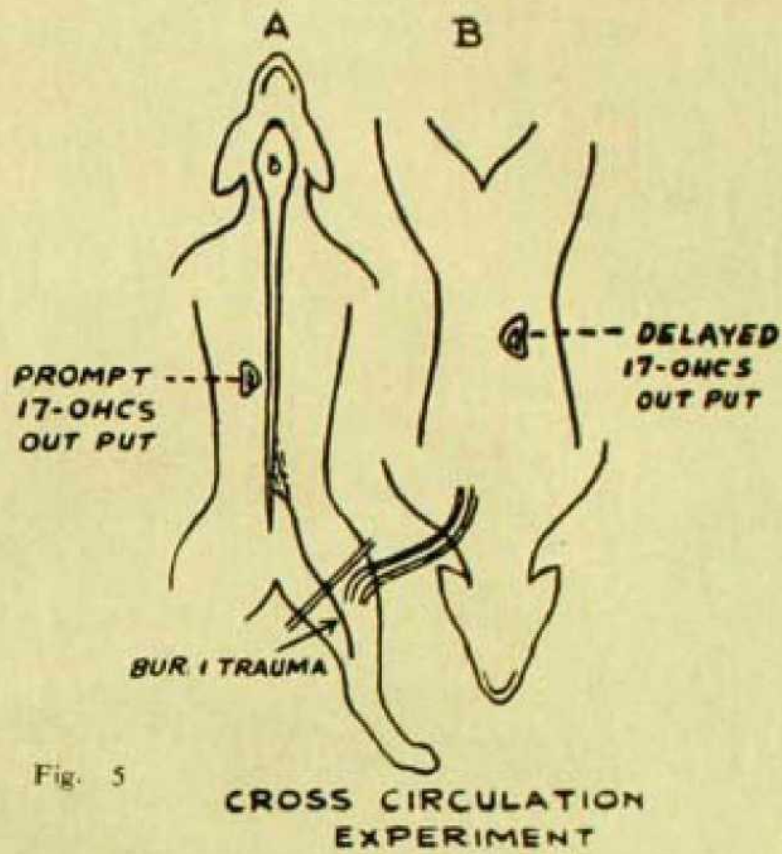


Fig. 5

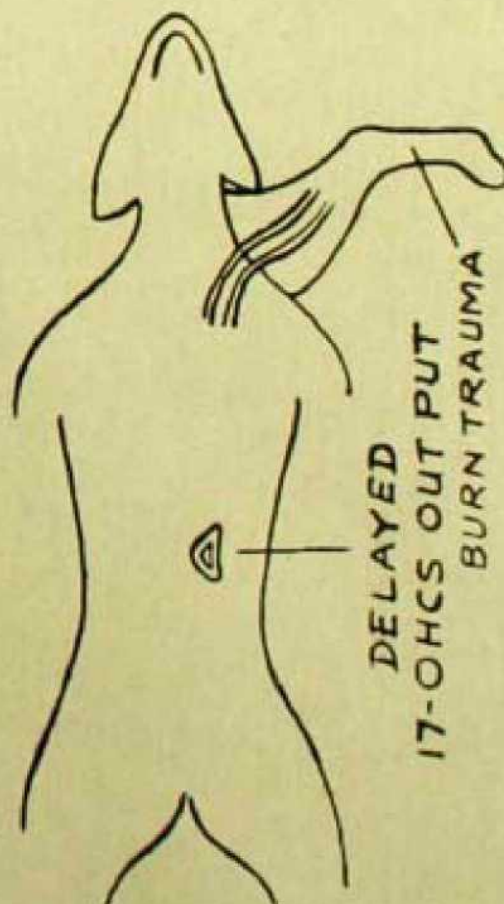


Fig. 4—Grafting experiment.



from the trunk, proximal to the anastomosis, keeping only the sciatic nerve intact. This is an acute experiment where we can study the influence of burn on the hind limb of dog A, receiving the cross circulation from dog B. Thus in dog A the pituitary-adrenal-axis will be influenced by nerves only and dog B will have the influence on the same axis only by vascular link. Both the dogs received 5% glucose, 5% glucose in saline as fluid transfusion. Heparin was used locally. Eight such experiments have been conducted. Cannulation of adrenal veins of both dogs A and B was performed after the method of Hume and Nelson (1955) before the experiment was started. Plasma 17-OHCS was estimated after the method of Silber and Porter (1954).

#### Group—VII :—

In this group the *grafting experiment* (Fig. No. 4) has been carried out by taking the disarticulated right hind limb of a dog and grafting it to the neck of another dog. Vascular communication was made by anastomosis of the femoral artery and vein with the external carotid artery and jugular vein respectively. Clotting was prevented by heparin. When the circulation was found to be established, standardised burn was inflicted on the grafted extremity. In this experiment no nerve is there and if any effect on the 17-hydroxycorticosteroid secretion by the adrenal is noted, that is accountable by the effect on the pituitary-adrenal-axis by chemicals from the burned extremity and mediated through the vascular path. Right adrenal vein was cannulated previously (48 hours) after the method of Hume and Nelson (1955) and Plasma 17-OHCS was estimated after the method of Silber and Porter (1954).

This type of experiment has got a draw-back that the dog will bleed into the grafted extremity with lowering of his blood volume and any observable effect on the adrenocortical secretion rate may be ascribed to this. But when the dog receives transfusion, this question, does not arise. And so 5% glucose, 5% glucose in saline transfusion was used throughout the whole procedure.

#### Group—VIII :—

Effect of burn on the pituitary-adrenal-axis of a dog whose entire brain has been taken out including the spinal cord (Fig. No. 5).

This experiment has been carried out with a view to study the pituitary-adrenal-axis in the absence of the total central nervous system structures but leaving behind the pituitary only and further, whether burn applied to the hind limb can influence the same axis.

Seven days before the experiment, removal of the splanchnics and the abdominal sympathetic ganglia was carried out.

The dogs were operated under nembutal anaesthesia. Brain removal was carried out through craniotomy openings by methods of section and suction. In connection with this operation we require to know the folds of the dura mater.

#### (a) *The falx Cerebri*

This is situated in the median plane between the two cerebral hemispheres. It is sickle shaped and the curve is gradual. The upper border



is attached to the midline of the cranium above, from the crista galli to the osseous tentorium. The falx contains the sagittal sinus. Its lower border is concave and free.

(b) *Tentorium Cerebelli*

It is situated between the cerebral hemispheres and the cerebellum. Its central part is bony and the lateral part is membranous. On to the projecting border of the temporal bone the lateral membranous part gets its attachment. The free edge of the tentorium bounds a space through which the middle and the posterior cranial fossae communicate.

(c) *Diaphragma Sellae*

It is a circular fold of dura mater surrounding the sella turcica having an opening in the centre and below it the pituitary is situated.

During the process of removal of the brain, the middle cerebral arteries stand out in prominence and these require ligation to minimise haemorrhage. In all the experiments (both here and previously stated) where brain removal operations have been performed, bleeding from vessels has been checked by the following procedures as required :—

(i) ligation, (ii) application of Cushing's clips, (iii) cautery and (iv) gelfoam.

The tentorium cerebelli was removed and the cerebellum was extirpated and this facilitated the removal of the hind brain. The pituitary was left alone (solitary Pituitary).

The spinal cord extends from the foramen magnum to the seventh lumbar vertebra. At the foramen magnum it is continuous with the medulla oblongata. The hind part of the spinal cord tapers rapidly to form the conus medullaris and the filum terminale is beyond it. The filum terminale is a very thin structure and composed of pia mater and small amount of nervous tissue. The terminal part of the spinal cord is surrounded by bunches of nerve roots called cauda equina.

Removal of the spinal cord was done by three or four laminectomies at different levels. The dura was opened and the cord was divided at the levels of laminectomies. These multiple laminectomies helped in the easy passage of snares for dividing the spinal nerves. Subsequently the divided parts of the spinal cord was removed by suction. The blood pressure came down to a low level. Artificial respiration was carried out. Recently drawn blood from hypophysectomized donors and 5% glucose, 5% glucose in saline were transfused.

The right adrenal vein was cannulated for collection of adrenal venous blood after the method of Hume and Nelson (1955) forty eight hours before the starting of the experiment. The blood samples were collected in heparinized tubes. The plasma was separated by centrifuging and preserved in cold. 17-hydroxy-corticosteroids were estimated after the method of Silber and Porter (1954). Standardized burn was inflicted on the right hind limb.

The pituitary was histologically studied by the staining method of Gomori's CAHP and haematoxylin and eosin stain.



*Group—IX—Splanchnicectomy with sympathectomy and burn*

*Nervous splanchnicus major* :—It starts from the 12th thoracic sympathetic ganglion and after entering the abdomen between the lumbar part of the diaphragm and the psoas minor muscle joins a small ganglion situated dorsal to the coeliac ganglion.

*Nervous splanchnicus minor* :—The lesser splanchnic nerve starts from the last thoracic and first lumbar ganglia and joins a plexus around the adrenal gland. This nerve may be double or treble.

*Abdominal part of the sympathetic system* :—The thoracic sympathetic nerve cord enters into the abdomen and then into the pelvis. There are seven lumbar ganglia on it, which are situated between the two psoas minor muscles.

The splanchnics were divided and the abdominal sympathetic chains were removed during the adrenal vein cannulation. Forty eight hours after, the burn experiments were conducted (right hind limb). Six male dogs of 10 to 13 kg. in weight were used in this experiment.

## RESULTS

*Group—I :—Burn injury applied to single hind limb (Table I and statistical Table 1 : Fig. No. 6)*

Surgery of cannulation of the lumbo-adrenal vein under nembutal anaesthesia led to high 17-OHCS output. Forty eight hours after cannulation this value came down to a significantly low level. After burn the resting 17-OHCS output was significantly high at 0.1% level during 1/2, 1, 3, 24 and 72 hours. Intravenous administration of ACTH in such dogs led to further rise in adrenal venous 17-OHCS output which was highly significant at 0.1% level.

*Group—II :—Burn injury applied to both hind limbs (Table 2 and statistical table ; 2 Fig. No. 7)*

The high adrenal venous 17-OHCS output came down to a basal level forty eight hours after cannulation. The more severe burn stress in this group manifested with significantly high 17-OHCS output at 1/2, 1, 3, and 6 hours after burn. At 6 hours there were only 4 observations. At 12 hours the corticosteroid output was significantly high at 5% level and the number of observations was 3 only as rest of the dogs died. At 24 hours only one dog survived and the 17-OHCS output was 31.000 mcg./minute. Corresponding observation in the same dog forty eight hours after cannulation was 1.7 mcg./minute. There are four other observations at 48 hours after cannulation with S. D. of the value of 0.837 with D.F. 4. Fiducial limits of 1.7 at 0.1% level are -5.504 to 8.904, i.e. 0 to 8.904. The value 31.000 is well beyond these limits. This means 31.000 is significantly different from 1.7. Intravenous ACTH at 12 hours after burn did not show any significant rise of 17-OHCS output over and above the already high level of it.



When the single extremity burn was compared to the double extremity burn, there was a significant difference at 1% level in the 17-OHCS output at 30 minutes after burn. At 24 hours the difference was significant only at 5% level (statistical table 16).

*Group—III :—Amputation of a hind limb (Table 3 and statistical Table 3 ; Fig. No. 8)*

Amputations of hind limbs lead to significantly high adrenal venous 17-OHCS output at 0.1% level when the values at 1, 3, 6 and 24 hours were compared to the basal post-cannulation value. Forty eight hours after amputation the output was very low and its difference with the post-cannulation value was significant only at 5% level. After intravenous ACTH at 48 hours the output rose to a significantly high level.

*Group—IV :—Burn and amputation*

(A) Amputation performed sixty minutes after burn (Table 4 and statistical Table 4 ; Fig. No. 9).

Five dogs were used in this set of experiment. Forty eight hours after cannulation the basal 17-OHCS output was significantly lower than the output noted during surgery and cannulation. Burn to the hind limb showed significantly higher level of 17-OHCS output at 30 and 60 minutes. Amputation of the burned extremity was performed 60 minutes after burn. Thereafter the steroid output had a tendency to fall and a significantly low value (0.1% level) was noted at 6 hours. The value further decreased at 48 hours. The adrenal cortex was well responsive as noted by the significantly high 17-OHCS output when intravenous ACTH was administered.

(B) Amputation performed 2 hours after burn (Table 5 and statistical Table 5 ; Fig. No. 10).

After the basal 17-OHCS output was achieved 48 hours after cannulation, burn trauma to the hind limb lead to significantly high 17-OHCS output upto 2 hours. At this period amputation of the burned limb was performed. At one hour after amputation the 17-OHCS output was significantly higher than that noted at 2 hours after burn. Two hours after amputation there was no difference. At 6 hours after amputation the fall was significant at 1% level. Maximum fall was noted at 48 hours after amputation. The adrenal cortex was well responsive to exogenous ACTH.

(C) Amputation performed 3 hours after burn (Table 6 and statistical Table 6 ; Fig. No. 11).

The post-cannulation 17-OHCS output rose to a significantly higher level after burn injury to a hind limb upto 3 hours. Amputation of the burned limb was performed 3 hours after burn trauma. Sixty minutes after amputation there was significantly high 17-OHCS output. At 6 hours after amputation there was no difference in the 17-OHCS output when compared to that at the third hour after burn. A significantly low output was observed at 24 and 48 hours after amputation. The adrenal cortex responded well to ACTH.



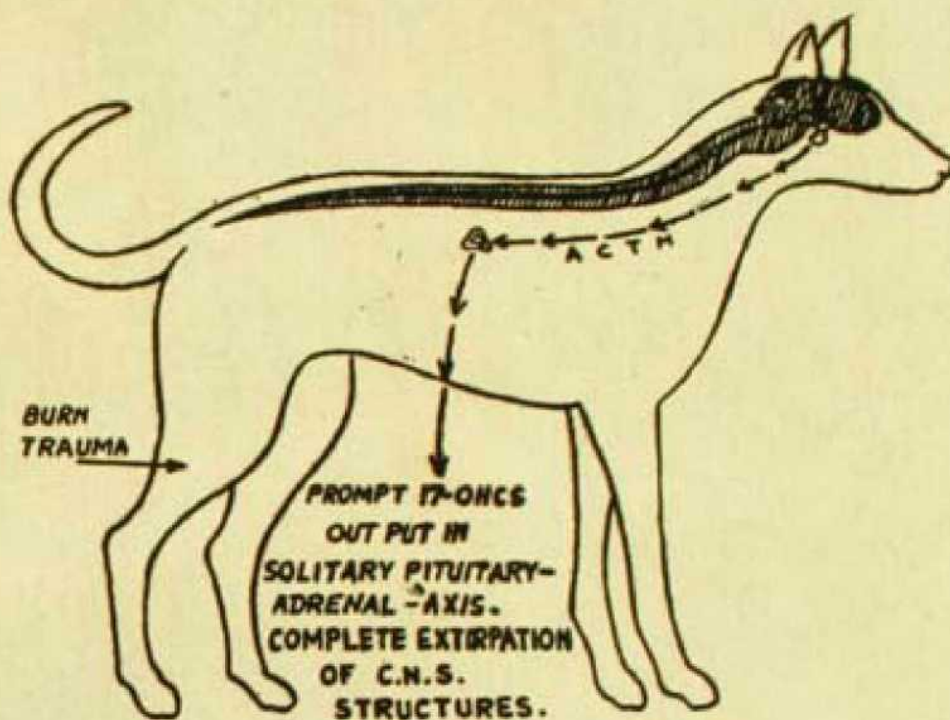


Fig. 5

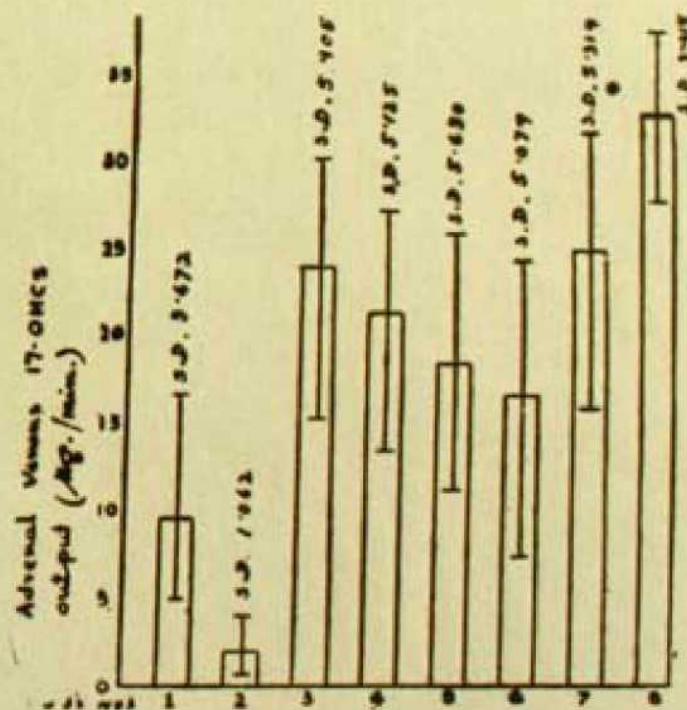


Fig. 6.—Effect of Burn (Single Extremity).



TABLE 1  
Effect of burn (single extremity) on dogs

Dog numbers Observation numbers	21	22	23	24	25	26	27	28	29
	Adrenal venous 17-OHCS output (micrograms/minute)								
1. During adrenal vein cannulation	..	5.0	9.5	8.0	12.5	11.7	16.9	15.5	13.2
2. Forty-eight hours after cannulation	..	1.5	0.7	1.2	4.0	0.9	3.5	3.5	3.0
3. Thirty minutes after burn	..	15.3	19.1	18.6	29.8	16.0	30.0	30.4	29.4
4. Sixty minutes after burn	..	13.6	18.4	13.8	27.1	15.4	27.3	26.5	19.0
5. Three hours after burn	..	12.0	13.2	14.1	21.0	10.5	27.1	21.6	25.0
6. Twenty-four hours after burn	..	7.4	14.3	9.0	20.0	11.6	24.8	21.1	17.0
7. Seventy-two hours after burn	..	16.0	23.4	17.6	32.2	18.0	30.1	30.0	31.3
8. ACTH (I.V.)	..	28.1	32.2	29.3	38.3	29.4	37.2	34.0	35.2



Table 1 (Contd.)

Dog numbers Observation numbers	20	31	32	33	34	35	Mean	S.D.	D.F.	
	Adrenal venous 17-OHCS output (micrograms/minute)									
1. During adrenal vein cannulation	..	6.2	5.4	10.0	6.9	9.8	6.3	9.640	3.672	14
2. Forty-eight hours after cannulation	..	0.8	1.6	2.8	2.0	1.5	1.8	2.047	1.062	14
3. Thirty minutes after burn	..	19.0	28.6	28.2	16.6	26.0	25.4	24.133	5.905	14
4. Sixty minutes after burn	..	16.4	23.4	27.0	21.3	22.7	23.6	21.540	5.125	14
5. Three hours after burn	..	15.5	20.0	25.3	11.2	22.7	18.0	18.747	5.630	14
6. Twenty-four hours after burn	..	12.0	19.3	21.7	17.9	16.0	21.2	16.900	5.079	14
7. Seventy-two hours after burn	..	24.6	27.0	25.5	20.0	27.3	25.0	25.247	5.319	14
8. ACTH (I.V.)	..	36.2	32.1	30.6	32.2	37.3	32.4	33.447	3.315	14



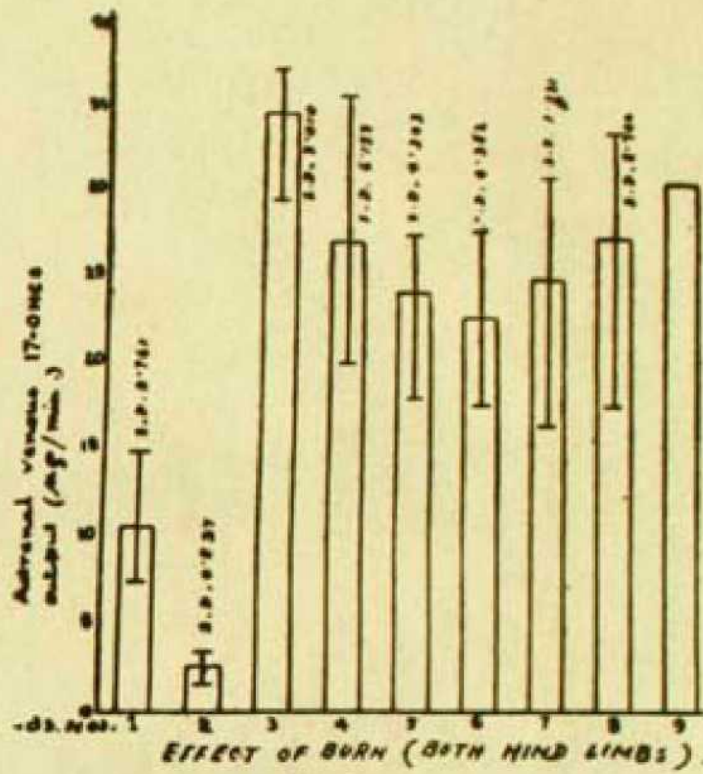


Fig. 7

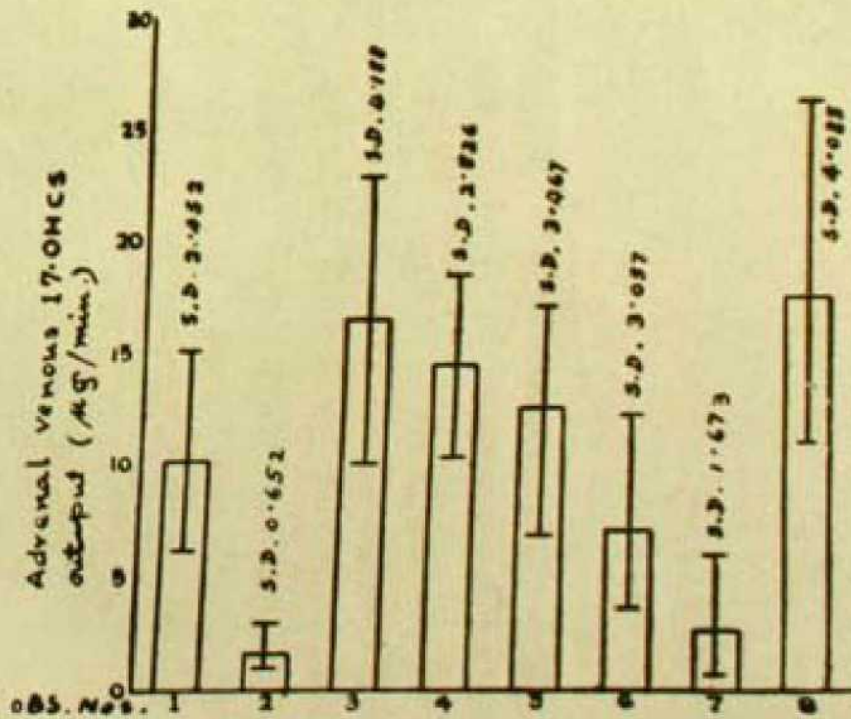


Fig. 8



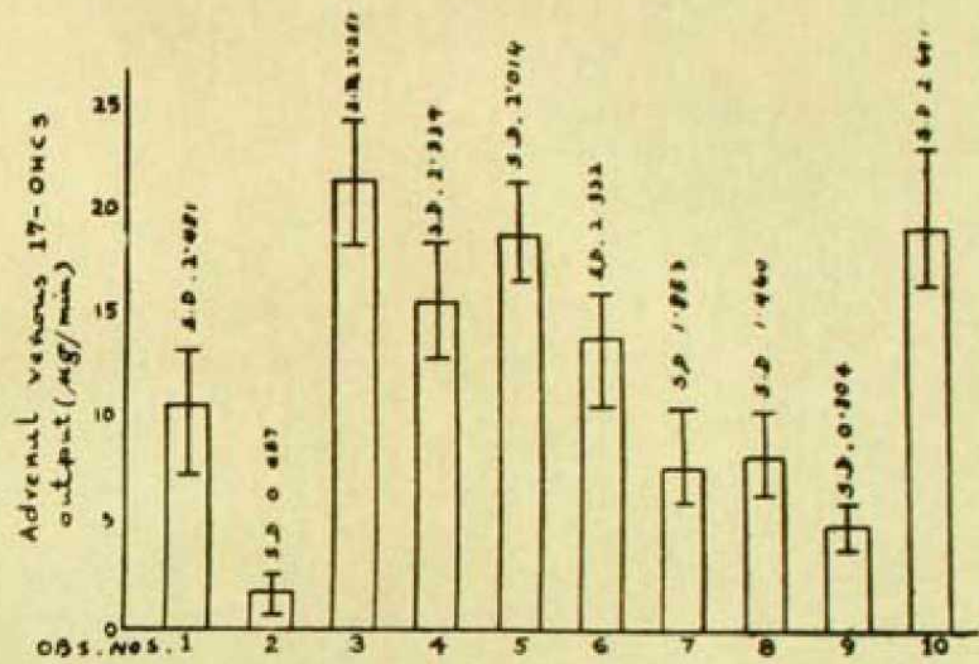


Fig. 9

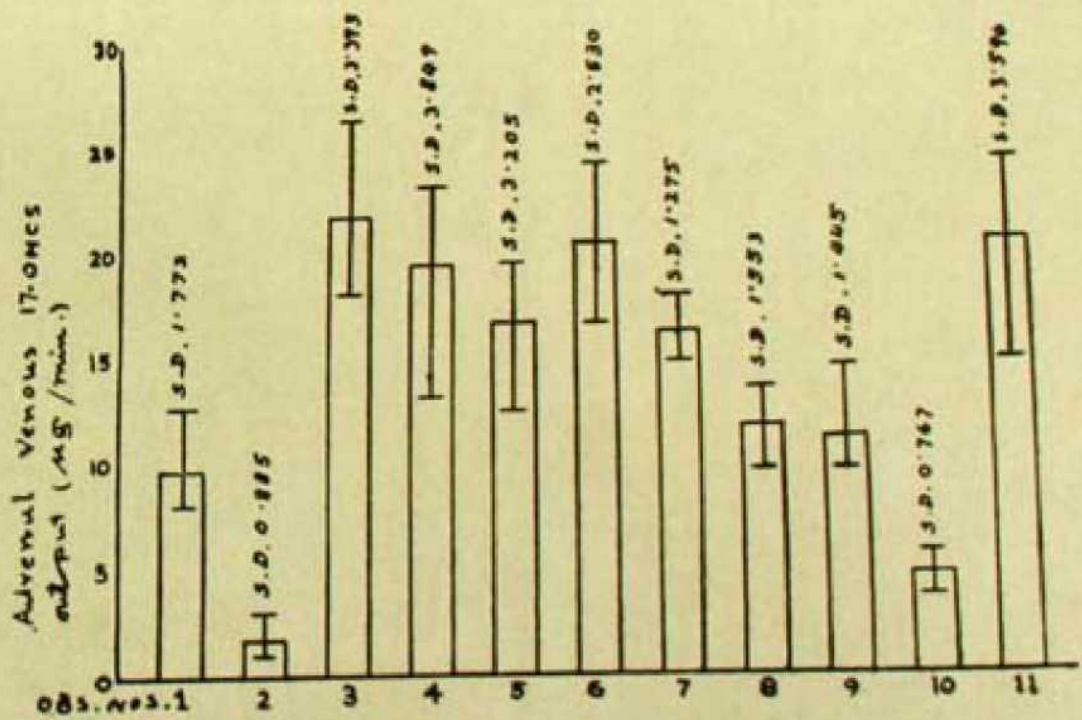


Fig. 10



TABLE 2  
Effect of burn (both hind limbs) on dogs

Dog numbers Observation numbers	36	37	38	39	40	Mean	S.D.	D.F.
	Adrenal venous 17-OHCS output (mic rograms/minute)							
1. During adrenal vein cannulation	14.9	8.0	10.2	7.3	12.1	10.500	2.761	4
2. Forty-eight hours after cannulation	1.7	3.1	2.9	1.5	3.3	2.500	0.837	4
3. Thirty minutes after burn	37.5	34.8	36.4	29.8	36.0	34.900	3.010	4
4. Sixty minutes after burn	36.0	25.5	24.2	20.0	30.4	27.220	6.153	4
5. Three hours after burn	27.8	25.0	18.0	22.1	28.4	24.260	4.303	4
6. Six hours after burn	28.0	21.6	Died	17.7	24.6	22.975	4.382	3
7. Twelve hours after burn	28.0	31.2		16.5	Died	25.223	7.731	2
8. ACTH (I.V.)	31.4	34.0		17.8		27.733	8.700	2
9. Twenty-four hours after burn..	31.0	Died		Died		1.000		







TABLE 3 (Contd.)

Dog numbers Observation numbers	50	51	52	53	54	55	Mean	S.D.	D.F.	
Adrenal venous 17-OHCS output (micrograms/minute)										
1. During adrenal vein cannulation	..	8.3	7.6	15.1	13.0	11.2	8.0	10.133	2.452	14
2. Forty-eight hours after cannulation	..	1.1	2.0	2.2	3.0	1.8	1.9	1.740	0.652	14
3. One hour after amputation	..	10.0	13.0	19.5	14.8	18.5	16.3	16.500	4.188	14
4. Three hours after amputation	..	12.5	12.3	16.6	18.2	12.6	12.0	14.500	2.826	14
5. Six hours after amputation	..	6.9	10.9	17.1	13.0	14.7	10.1	12.607	3.467	14
6. Twenty-four hours after amputation	..	4.4	4.9	12.2	10.0	6.0	7.2	7.000	3.057	14
7. Forty-eight hours after amputation	..	0.9	1.7	6.0	4.5	2.1	3.0	2.700	1.673	14
8. ACTH (I.V.)	..	12.9	13.5	21.0	26.5	20.4	16.6	17.553	4.085	14



TABLE 4  
*Effect of amputation of the burned limb. Amputation performed one hour after burn*

Dog numbers Observation numbers	56	57	58	59	60	Mean	S.D.	D.F.
	Adrenal venous 17-OHCS output (micrograms/minute)							
1. During adrenal vein cannulation	12.5	11.6	7.2	9.1	13.1	10.700	2.481	4
2. Forty-eight hours after cannulation	2.0	0.8	1.7	2.0	2.6	1.820	0.657	4
3. Thirty minutes after burn	22.0	21.3	18.1	21.3	24.4	21.420	2.251	4
4. Sixty minutes after burn	14.1	15.5	12.9	17.6	18.5	15.720	2.339	4
5. One hour after amputation	17.5	18.5	16.6	20.5	21.4	18.900	2.014	4
6. Three hours after amputation	10.6	15.3	12.4	15.4	16.0	13.940	2.332	4
7. Six hours after amputation	6.5	8.1	6.0	10.7	7.2	7.700	1.853	4
8. Twenty-four hours after amputation	7.6	10.4	6.4	8.2	8.5	8.220	1.460	4
9. Forty-eight hours after amputation	6.0	5.6	4.8	3.9	5.1	5.080	0.804	4
10. ACTH (I.V.)	16.6	17.4	20.5	18.7	23.2	19.280	2.641	4



TABLE 5  
*Effect of amputation of the burned limb. Amputation performed two hours after burn*

Dog numbers Observation numbers	61	62	63	64	65	66	Mean	S.D.	D.F.
	Adrenal venous 17-OHCS output (micrograms/minute)								
1. During adrenal vein cannulation	..	9.5	8.2	10.7	12.8	9.8	9.833	1.772	5
2. Forty-eight hours after cannulation	..	1.5	1.9	2.9	3.0	1.3	1.933	0.885	5
3. Thirty minutes after burn	..	20.5	25.4	26.4	21.6	18.0	21.833	3.393	5
4. Sixty minutes after burn	..	16.6	23.2	21.1	20.7	21.9	19.417	3.849	5
6. Two hours after burn	..	18.2	19.6	19.5	17.9	13.1	16.783	3.205	5
6. One hour after amputation	..	21.6	24.3	20.0	21.0	16.6	20.533	2.530	5
7. Three hours after amputation	..	15.5	17.0	18.0	16.8	15.0	16.183	1.275	5
8. Six hours after amputation	..	10.4	13.4	13.2	12.3	9.5	11.800	1.553	5
9. Twenty-hour hours after amputation	..	13.5	11.5	9.6	11.6	10.0	11.067	1.445	5
10. Forty-eight hours after amputation	..	5.8	4.2	4.8	5.4	3.7	4.800	0.767	5
11. ACTH (L.V.)	..	20.5	24.0	21.7	24.4	14.8	20.683	3.590	5



TABLE 6  
*Effect of amputation of the burned limb. Amputation performed three hours after burn*

Dog numbers Observation numbers	67	68	69	70	71	Mean	S.D.	D.F.
	Adrenal venous 17-OHCS output (micrograms/minute)							
1. During adrenal vein cannulation	12.4	9.0	7.8	11.1	10.1	10.080	1.788	4
2. Forty-eight hours after cannulation	2.7	2.5	1.8	3.0	1.9	2.380	0.517	4
3. Thirty minutes after burn	24.0	18.6	16.8	22.2	20.8	20.480	2.852	4
4. Sixty minutes after burn	18.2	14.9	20.3	26.7	16.0	19.220	4.669	4
5. Two hours after burn	14.6	11.6	14.4	19.0	15.1	14.940	2.508	4
6. Three hours after burn	16.5	10.8	19.2	18.0	16.0	16.100	1.018	4
7. One hour after amputation	21.4	17.0	28.1	24.5	21.4	22.480	4.124	4
8. Three hours after amputation	17.0	15.4	20.2	18.6	17.8	17.800	1.789	4
9. Six hours after amputation	14.8	12.4	20.0	17.5	16.6	16.300	0.883	4
10. Twenty-four hours after amputation	9.8	8.2	10.6	13.4	10.7	10.540	1.886	4
11. Forty-eight hours after amputation	4.5	3.1	4.3	6.1	4.3	4.460	1.071	4
12. ACTH (I.V.)	18.0	16.7	21.5	19.4	19.7	19.060	1.815	4



TABLE 7  
*Subtotal isolation of the limb (femoral artery, vein and sciatic nerve intact) and burn*

Dog numbers Observation numbers	72	73	74	75	76	Mean	S.D.	D.F.
	Adrenal venous 17-OHCS output (micrograms/minute)							
1. During adrenal vein cannulation ..	8.0	11.2	9.8	14.5	10.2	10.740	2.400	4
2. Forty-eight hours after cannulation ..	0.9	3.2	1.5	2.0	3.1	2.140	1.001	4
3. Thirty minutes after isolation of hind limb ..	10.8	20.4	12.7	15.3	12.0	14.240	3.818	4
4. Sixty minutes after isolation of hind limb ..	9.4	15.6	10.2	11.5	10.7	11.480	2.426	4
6. Thirty minutes after burn ..	20.7	30.0	21.9	24.0	20.3	23.380	3.971	4
6. Sixty minutes after burn ..	16.0	28.9	16.4	27.0	18.2	21.300	6.164	4
7. Three hours after burn ..	14.5	22.1	11.2	24.6	17.1	17.900	5.468	4
8. ACTH (Subcutaneous injection into the burned limb) ..	22.2	28.7	19.0	27.1	25.4	24.480	3.897	4



TABLE 8  
*Subtotal isolation of the limb (femoral artery and vein intact) and burn. Sciatic neurectomy performed during isolation of the limb*

Dog numbers Observation numbers	77	78	79	80	81	82	83	84	Mean	S.D.	D.F.
	Adrenal venous 17-OHCS output (micrograms/minute)										
1. During adrenal vein cannulation	..	6.5	7.4	8.4	8.0	6.8	8.5	9.1	8.113	1.219	7
2. Forty-eight hours after cannulation	..	2.5	2.8	1.2	2.0	1.9	2.6	1.6	2.125	0.547	7
3. Thirty minutes after isolation of the hind limb.	13.4	9.5	12.2	10.6	8.9	9.0	10.0	12.4	10.750	1.710	7
4. Sixty minutes after isolation of the hind limb.	12.5	10.9	9.4	8.1	11.5	7.0	9.3	9.6	9.788	1.796	7
5. Thirty minutes after burn	..	10.7	11.0	9.2	10.0	8.5	8.3	10.0	10.150	1.662	7
6. Sixty minutes after burn	..	12.6	8.1	9.0	11.4	12.2	14.7	20.0	12.838	3.741	7
7. Three hours after burn	..	16.4	17.2	13.0	18.1	15.0	18.7	20.3	17.200	2.351	7
8. ACTH (subcutaneous injection into the burned limb).	26.4	24.5	20.0	20.5	24.9	23.4	27.5	28.0	24.400	2.986	7



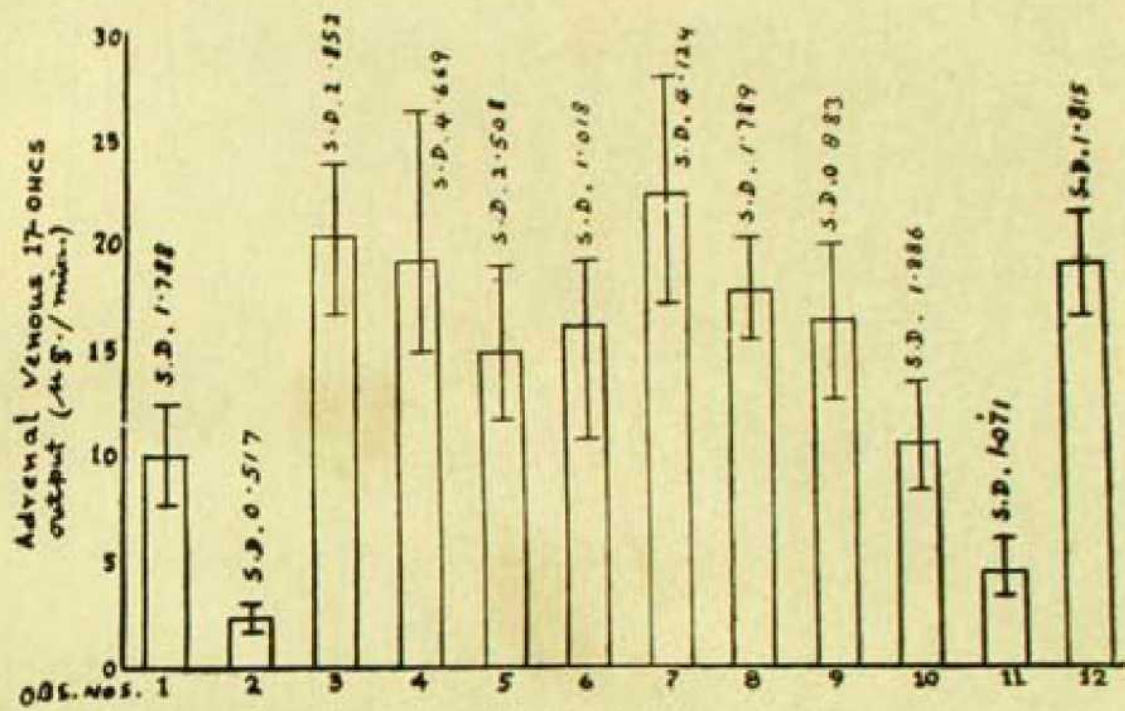


Fig. 11

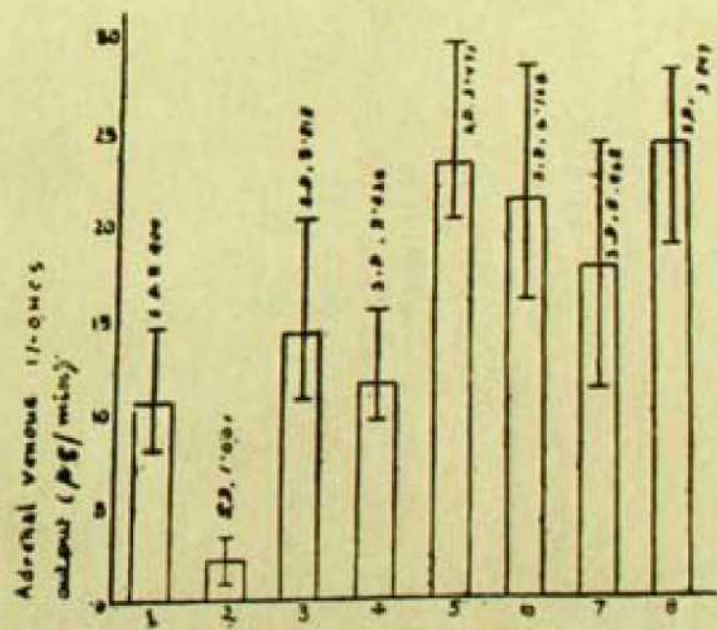


Fig. 12



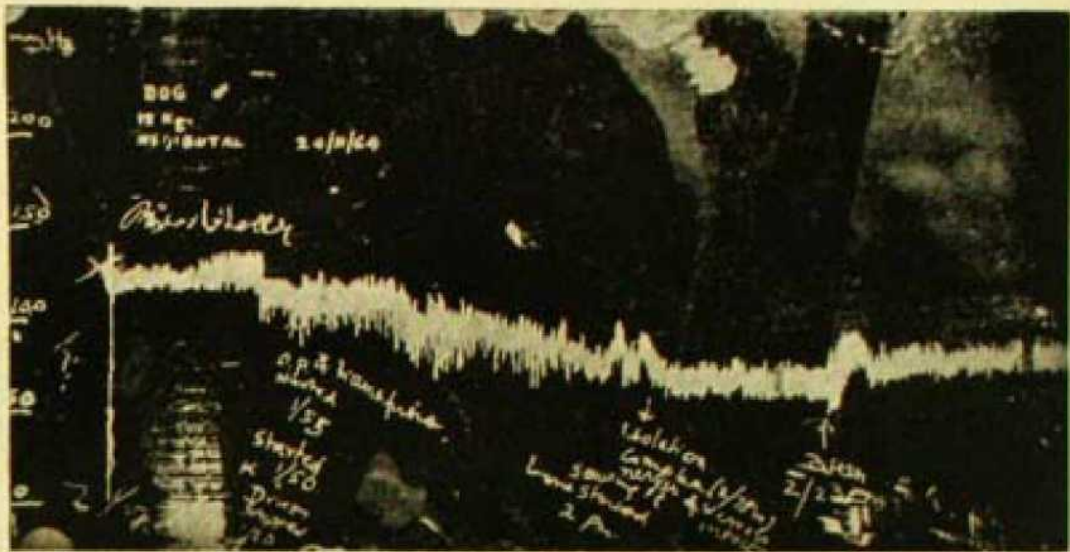


Fig. 13—Blood pressure changes during subtotal isolation of the hind limb and the effect of burn with neuro-vascular link intact.

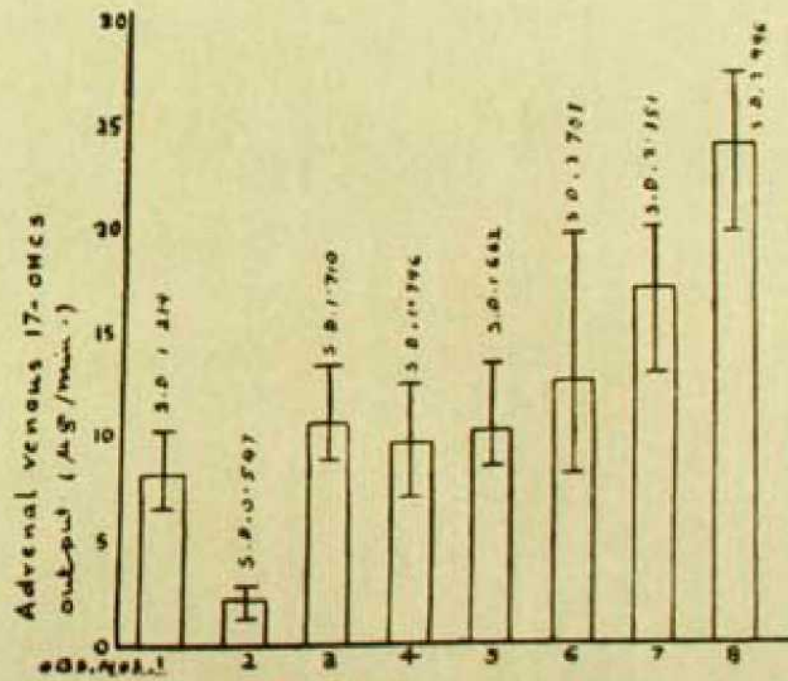


Fig. 14



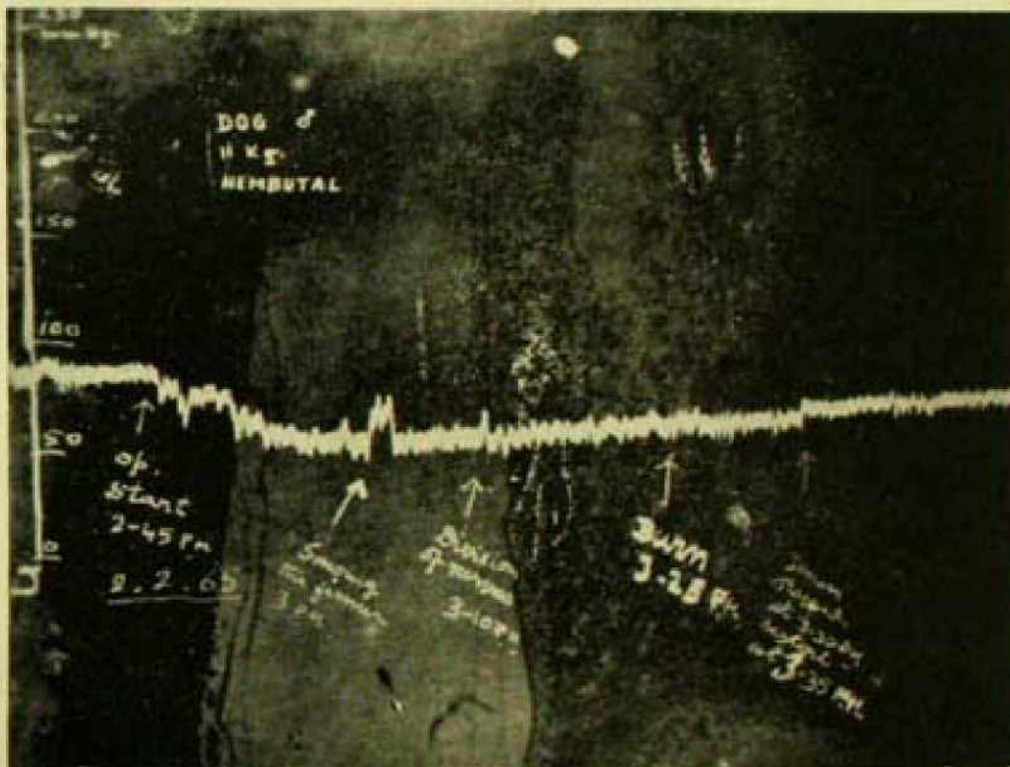
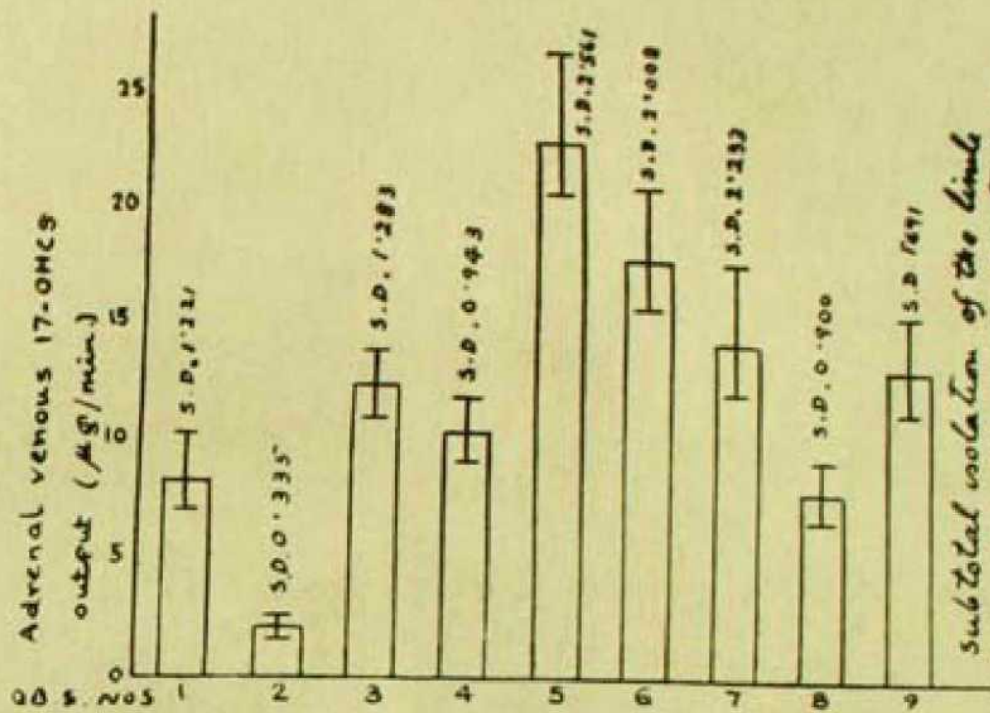


Fig. 15. Blood pressure changes during subtotal isolation of the hind limb and the effect of burn with vascular link intact.





*Subtotal isolation of the limb  
(Femoral artery, vein & sciatic nerve  
intact) and burn. Sciatic  
neurectomy performed 30 min-  
utes after burn.*

*Fig. no. - 19. 16.*

Fig. 16

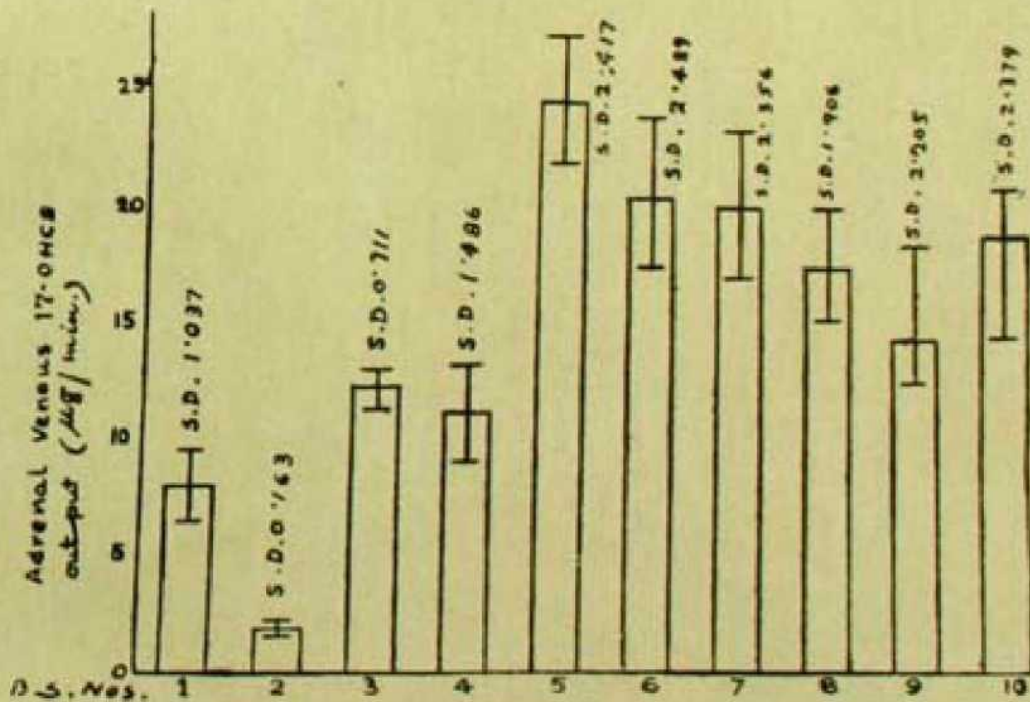


Fig. 17



*Group—V :—Acute isolated limb experiment*

- (A) Subtotal isolation of the hind limb keeping the neurovascular link intact (Table 7 and statistical Table 7 : Fig. No. 12).

The basal 17-OHCS output forty eight hours after cannulation reached a high level when surgery to achieve this subtotal isolation preparation was performed. Burn to such an extremity manifested with a very prompt rise in 17-OHCS output. The adrenals were well responsive to exogenous ACTH administered locally.

The blood pressure change is recorded in (Fig. No. 13).

- (B) Subtotal isolation of the hind limb keeping only the vascular link intact (Table 8 and statistical Table 8) (Fig. No. 14).

Thirty and sixty minutes after such a preparation the adrenal venous 17-OHCS output was significantly higher than the basal output noted at 48 hours after cannulation. After three hours of burn injury to such an extremity there was highly significant rise in 17-OHCS output at 0.1% level. Previous to this period there was no significant rise in 17-OHCS output.

The blood pressure change is recorded in fig. No. 15.

- (C) Subtotal isolation of the hind limb keeping the neurovascular link intact. Neurectomy performed 30 minutes after burn (Table 9 and statistical Table 9) (Fig. No. 16).

The 17-OHCS output was significantly high after such a preparation. Thirty minutes after burn injury of the same extremity the output was further elevated. Highly significant lowering of the adrenal venous 17-OHCS output occurred at 30, 60 and 180 minutes after neurectomy when it was done, 30 minutes after burn. Exogenous ACTH administered locally showed a good response.

- (D) Subtotal isolation of the hind limb, keeping the neurovascular link intact. Neurectomy performed 60 minutes after burn (Table 10 and statistical Table 10) (Fig. No. 17).

In this group the surgery for such a preparation led to high 17-OHCS output and burn trauma to such an extremity produced further rise. Neurectomy was performed 60 minutes after burn. Thirty minutes after neurectomy there was no significant difference in the 17-OHCS output when compared to that at 60 minutes after burn. The value gradually came down and at 3 hours after neurectomy there was a significant lowering. ACTH injected locally led to further rise in 17-OHCS output.

- (E) Subtotal isolation of the hind limb, keeping the neurovascular link intact. Neurectomy performed 180 minutes after burn (Table 11 and statistical Table 11) ; (Fig. No. 18).

Surgery for such a preparation manifested with increased 17-OHCS output and burn trauma to the same hind limb stimulated the pituitary-adrenal-axis further. When the neurectomy was performed three hours after burn, no highly significant fall was noted but at sixty minutes the significance was at 5% level. Exogenous ACTH injected locally stimulated the adrenal cortex further.



*Group—VI :—Cross circulation experiment ; (Table 12 and statistical Table 12) (Fig. No. 19.)*

In the recipient dog (R) *i.e.* dog A, the adrenal venous 17-OHCS output was significantly high at 30, 60 and 120 minutes after burn. Gradually there was a tendency to fall.

In the donor dog (D) *i.e.* dog B, there was a late rise in 17-OHCS output.

*Group—VII :—Burn trauma to the grafted limb (Table 13 and statistical Table 13 and 17) ; (Fig. No. 20).*

The basal adrenal venous 17-OHCS output was significantly high after grafting of the hind limb. Burn trauma to such an extremity led to a gradual rise in 17-OHCS output. The maximum rise noted was at 3 hours after burn. ACTH injected subcutaneously into the grafted limb stimulated adrenals proving thereby that absorption from the burned limb occurs. The increased response was noted within 1/2 an hour.

When the response of the adrenals after burn in this group was compared to that in the single extremity burn group (statistical Table 17), a highly significant difference (0.1% level) was observed in the thirty minutes' observation. It was significant at 1% level in sixty minutes' observation. At three hours there was no difference.

*Group—VIII :—Ablation of all central nervous system structures including the spinal cord, leaving behind the pituitary only and burn (Table 14 and statistical Table 14) ; (Fig. No. 21).*

The operation of removal of the brain and the spinal cord produced a good amount of shock to the animals and transfusion was an essential requirement. After isolation of the pituitary, 17-OHCS output was low at 30 and 60 minutes. When rest for 3 hours was allowed, the steroid output was high and burn trauma applied to a hind limb led to significant rise in 17-OHCS output at 1% level in 30 minutes and at 0.1% level at 1 and 2 hours. Upto 3 hours after burn the rise was significant. The adrenals were responsive to exogenous ACTH. Histologically the pituitary did not manifest any gross damage either in the cytoarchitecture or in vascularity.

*Group—IX :—Effect of burn on splanchnicectomized and sympathectomized dogs (Table 15 and statistical Table 15) ; (Fig. No. 22).*

Splanchnicectomy and sympathectomy did not block the increased 17-OHCS output after burn injury to the hind limb. The 17-OHCS output was significantly high 30 minutes after burn. Forty eight hours after burn the output remained at a high level and intravenous ACTH could stimulate the adrenals further.

## DISCUSSION

Burn injury to single or both hind limbs of the dog is a very severe stress leading to increased activity of the pituitary-adrenal-axis. The augmented activity has been studied upto 72 hours in dogs with single extremity burn and there is only one observation upto 24 hours in dogs



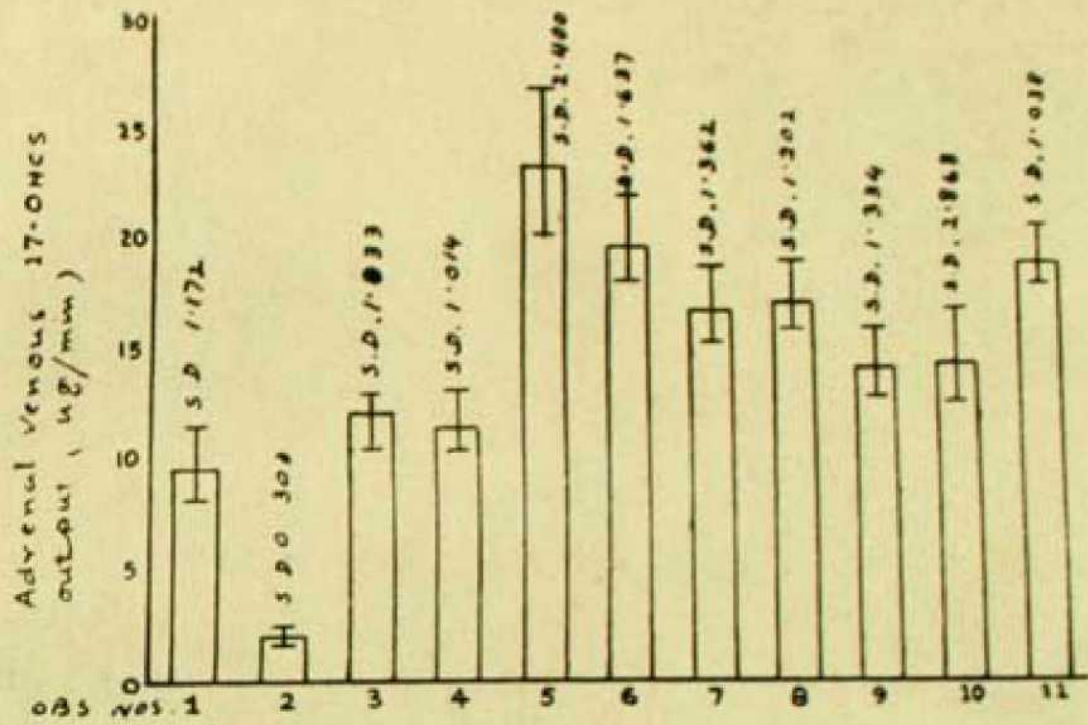


Fig. 18

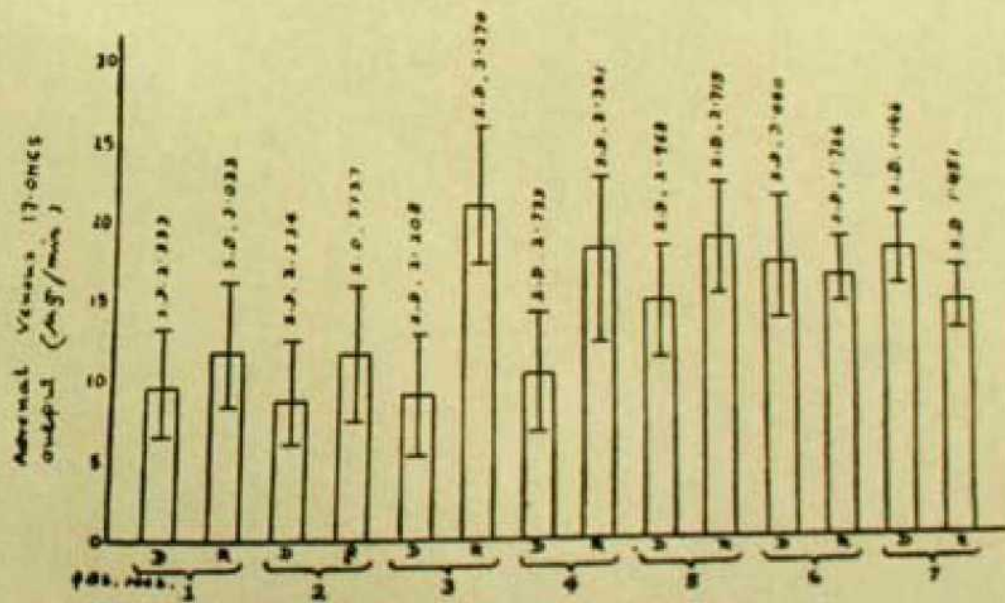


Fig. 19



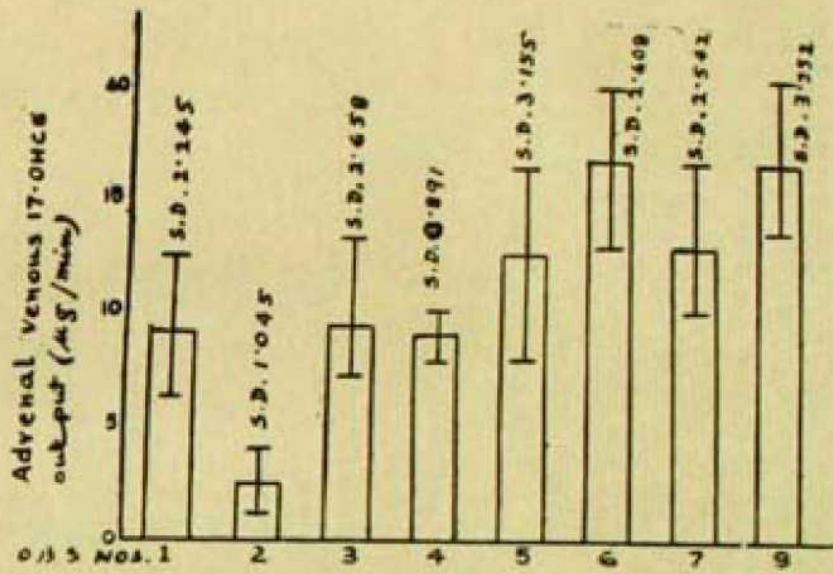


Fig. 20

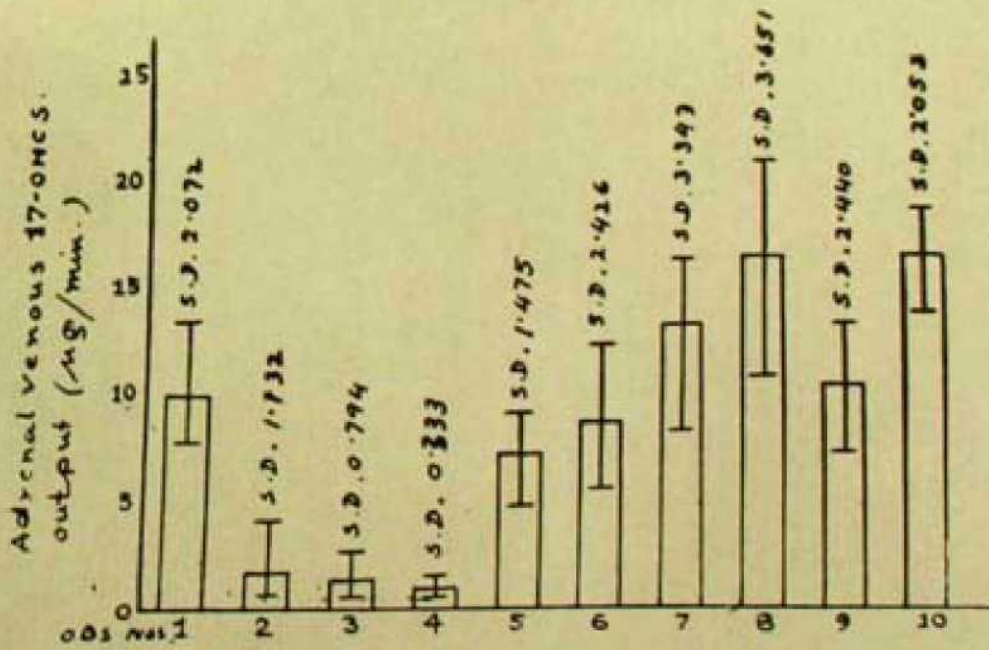
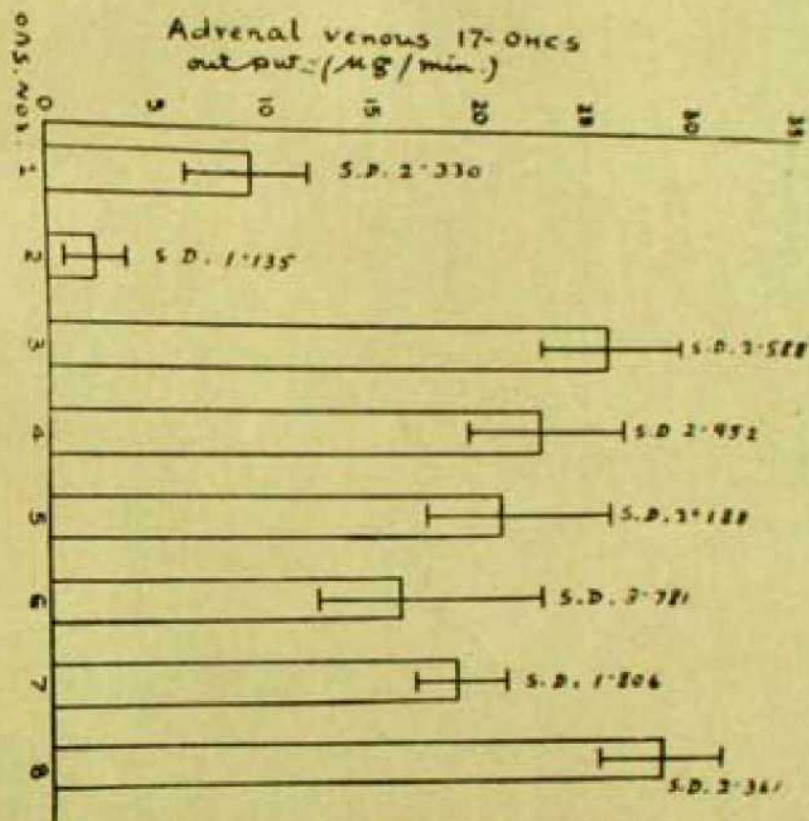


Fig. 21





EFFECT OF BURN ON SPLANCHNICECTOMIZED  
AND PARTIALLY SYMPATHETOMIZED  
DOGS.

Fig. 22



with burn of both hind limbs as the other dogs died before this period. This proves that the burn injury in the latter group is more serious than in the former one. However, when we compare the 17-OHCS output in these two groups, it is noted that the adrenocortical response is more or less same except at 30 minutes and at 24 hours. At these periods dogs of the latter group manifest with more increased adrenocortical response. Evans and Butterfield (1951) found the fall in the eosinophil cell count to be closely related to the extent of the burn trauma and the fall was nearly complete in 40 to 60% burns. Wight *et al.* (1953) found no correlation between adrenocortical response and the extent of burn except in minor injuries. Sevitt (1951, 1954) noted that the eosinopenia was present only for 1 or 2 days in patients with burns of less than 10% of the body surface but in more severely burned subjects the eosinopenia lasted for 3 to 5 days. Roy (1953) observed that in burns affecting lesser body surface, the urinary corticosteroids were proportionately higher but not upto the standard that was found in moderately or severely burned patients in whom no difference in the raised adrenocortical activity could be made. Hardy (1955) made similar observations and said that both moderate and severe burns responded in a similar way regarding the excretion of corticoids. Hume *et al.* (1956) observed that patients with more than 45% body surface burn showed a greater excretion of urinary corticosteroids than those with less extensive burns. Blood corticoid level showed a similar difference. In the present study the type of burn that we have used is a very severe stress and so there was no significant difference in the increased adrenocortical activity except at 30 minutes and 24 hours.

When ACTH is administered intravenously in dogs with double extremity burn at 12 hours after the injury, there is no significant rise of 17-OHCS output over the already increased output, but in single extremity burn after 72 hours there is a significant rise in the output. This proves that when the adrenal has been maximally stimulated by the volleys of nerve impulses from the burned site, it can not be stimulated furthermore. However, after 72 hours when some adaptation has developed exogenous ACTH can further stimulate the adrenal cortex.

17-OHCS output in the single extremity burn group at 1/2, 1, 3, 24 and 72 hours after burn is high and this corroborates the observations of Rennels and Timmer (1962) on the effect of scalding on plasma levels of corticosterone in the rat. At 24 hours after scalding the plasma corticosterone level is still elevated, although a return towards the control level is seen. In our series 72 hours after burn trauma the value further tends to rise because of the superimposition of infection and possibly of the absorption of burn toxins.

Amputation of a limb is a stressing procedure and this leads to increased adrenal venous 17-OHCS output. Significantly high output is observed upto 24 hours. At 48 hours the difference was significant only at 5% level. Exogenous ACTH can stimulate the adrenals further.

Vaccaressa (1922), Christophe (1939), Rydigier (1888) and Salvioli (1891) found good results after exclusion or excision of the burned part. In the present study we want to know how long after amputation of the burned extremity the pituitary-adrenal-axis normalises. So we have



divided our experiment group into three different subgroups. The amputation has been performed at 60, 120 and 180 minutes after the burn injury. When the amputation has been performed one hour after burn, the raised 17-OHCS output after burn has a tendency to fall and a highly significant low value (0.1% level) is noted at 6 hours after amputation. When the amputation has been performed 2 hours after burn, the fall in the steroid output is significant at 1% level 6 hours after amputation. At this hour (6th hour) there is no fall in 17-OHCS output when the amputation has been performed 3 hours after burn. In all the groups the output is low at 48 hours. From these observations we find that quick amputation of the burned extremity tries to bring down the high 17-OHCS output after burn.

Egdahl (1959) said that adrenocortical stimulation after severe burns or operative trauma was dependent exclusively upon peripheral nerve impulses and he found no evidence for the production of *wound hormone* which could stimulate the pituitary-adrenal-axis. He used subacute and chronic preparation. The burn trauma was produced in dogs by the use of a heat lamp held just above the skin for 5 minutes. In the present study acute experiments have been carried out as we want to avoid oedema of the limb and thrombosis of the vessels. These have occurred quite frequently in our hands in chronic preparations. Moreover, we have produced burn trauma by immersing the subtotally isolated extremity in boiling water at 100°C for 30 seconds. From the results it is noted that when the neurovascular link is kept intact, there is prompt adrenocortical response after burn. When the neural link is divided keeping only the vascular link intact, highly significant increase in adrenocortical response is noted 3 hours after burn (statistical table 18). This proves that the burned area produces some chemicals which can activate the pituitary-adrenal-axis in the absence of the neural link. Absorption of ACTH injected locally can increase 17-OHCS output proving thereby that absorption from the burned area can occur. Recently Fox (1962) prepared saline extracts from flame-burned, scalded or normal mouse skin. These extracts were lethal after intravenous or intraperitoneal injection. They have been fractionated into two components. One is thromboplastic and the other not. Chaet (1962) demonstrated burn toxins in invertebrates. At least two toxins were present in the coelomic fluid of scalded starfish. One toxin was a cytotoxin and nondialyzable and the second toxin was responsible for inducing autotomy. Simonart (1962) studied the protection against a lethal burn in rabbits pretreated with phosphates or with burn oedema fluid. Protection afforded by the phosphates was only a partial one. Injection of burn oedema fluid into normal rabbits produced all the symptoms manifested by burned rabbits. Toxic elements present in the burn oedema produced antibodies in normal animals. A certain number of such rabbits were definitely protected from the lethal effect of burn. Dobrkovsky *et al.* (1962) said that antibodies in the burned organism neutralized toxic products of burned skin. Feodorov and Skurkovich (1962) discussed about the immunotherapy of burn sickness. Rosenthal *et al.* (1962) demonstrated the presence of toxin in the blood of acutely burned human subjects. Antitoxic-like substance in the blood





of healed burned subjects was also demonstrated. Preliminary clinical evidence suggested a beneficial and detoxifying effect of blood or plasma from healed burned subjects in very badly burned individuals. Malm and Slawikowski (1962) evaluated the different types of convalescent burn sera in the rat. A consistent protective effect was observed in several series of severely burned rats. The other reviews on burn toxin have been made previously. These prove that there are burn toxins. The late rise in 17-OHCS output after burn may be due to the absorption of the burn toxins from the isolated limb when only the vascular link remains intact.

Egdahl (1959) observed that the adrenal venous corticoid outputs per minute fell rapidly to basal levels within 20 to 45 minutes after neurectomy of the burned limb. However, from our observations we note that the fall is not to the basal level and moreover there is a difference in the amount of fall when the timing of neurectomy after burn varies. When the neurectomy is performed within 30 minutes after burn, highly significant lowering of the adrenal venous 17-OHCS output occurs at 30, 60 and 180 minutes after neurectomy and this is never to a basal level. There is significant lowering of the 17-OHCS output at 60 and 180 minutes when the neurectomy is performed 60 minutes after burn. When the neurectomy is performed 180 minutes after burn, there is no significant fall in adrenal venous 17-OHCS output except at 60 minutes where the significance is at 5% level. Thus we have not noted the basal level after neurectomy and the amount of fall is variable depending upon the interval of neurectomy after burn. This may be explained by the following two ways. (1) When the pituitary-adrenal axis has been triggered to a heightened activity, quick return to the basal level is retarded, and (2) absorption of burn toxins remains unimpeded and so they may act on the same axis.

In the type of cross circulation experiment that has been conducted here, the influence of the neural or vascular link over the pituitary-adrenal-axis can be separately assessed. Care should be taken to keep the blood pressure of the two animals at a reasonably standard level. The blood volume is also to be maintained. For this purpose we have used fluid transfusion. It is our experience that if the blood pressure falls below 35 mm. of Hg., the adrenal blood flow is sluggish and the corticoid output falls. The same opinion has been expressed by Hume and Nelson (1955). Frank *et al.* (1955) found that when adrenal blood flow was less than 17% of the control value, the hormonal output could no longer be maintained. In none of our observations the pressure was so low. Therefore observations on the 17-OHCS output in the two animals will truly reflect the status of the adrenocortical activity when burn trauma is inflicted to the cross-circulated limb. In these experiments we have observed a very rapid rise in 17-OHCS output in the dog whose hind limb is neurally connected to the remaining part of the body after application of burn trauma to this limb. The same stimulus leads to a delayed rise in 17-OHCS output in the dog getting the products of burn through systemic circulation and this can be explained by the action of burn toxins on the pituitary-adrenal axis.



In the grafting experiments pooling of blood in the grafted limb has been adjusted by transfusion. There is delayed rise in 17-OHCS output after application of burn trauma to the grafted limb. This may be due to absorption of burn toxins and their effect on the pituitary-adrenal-axis.

Lapchinsky (1960) described the results of experimental transplantation of preserved limbs and kidneys and possible use of this technique in clinical practice. He noticed the general effect of a toxic phenomenon after connecting an extremity to the systemic circulation of a dog. There was hurried respiration and increased pulse rate and a fall in blood pressure. Death occurred within 5 to 12 hours after recirculation. No toxic effects were observed when there was thrombosis in the anastomosed vessels. Eiken *et al.* (1964), however, observed no evidence of any toxic effect (changes in blood pressure, pulse rate and respiration) in any instance. These toxic effects were not even noted after recirculation of limbs kept for 24 hours at room temperature. All these animals survived and there were no ill effects.

Stress of burn applied to the hind limb of splanchnicectomized and partially sympathectomized dogs leads to the usual type of increased adrenocortical activity. This proves that adrenomedullary secretion is at least not essential for the increased adrenocortical activity after stress in dogs. The same opinion has been expressed by Hume and Nelson (1955) using haemorrhagic shock.

Adrenaline and noradrenaline have been studied by Goodall and Haynes (1960) and Goodall *et al.* (1957) in thermal burns. Goodall (1962) studied these in burned subjects. In surviving patients after burn injury the catecholamine excretion was high for 2 weeks to 3 months depending upon the amount of trauma and recovery of the patients. With recovery the levels came towards normal. Regarding the adrenaline content of the adrenal glands in patients who died, there was either normal or below normal level. Egdahl (1959) and Nykiel and Glaviano (1961) observed marked adrenomedullary stimulation after endotoxin injection. The latter authors noted prevention of increased catecholamine release after splanchnicectomy. They noted the rise only when there was a significant fall of arterial blood pressure. Hokfelt *et al.* (1962) noted increased secretion of adrenaline and noradrenaline after lethal doses of endotoxin in cat. The adrenal medulla continued to secrete increased catecholamines and there was no decreased activity of the sympathetic nervous system and the adrenal medulla. They also found that this release depended upon the neurogenic mechanism and partly due to a fall in arterial blood pressure. Egdahl (1960) noted low resting level of catecholamine output in dogs with isolated pituitary and there was increased response after burns.

Egdahl (1960) mentions that the center for control of adrenomedullary secretion is situated between C<sub>1</sub> and pons in dog and further that centers above the medulla is not required for maximum adrenomedullary response after trauma. Hess (1954) von Euler and Folkow (1958) and Ferguson *et al.* (1957) noted that stimulation of the different central nervous system structures resulted in increased adrenomedullary secretion.



Increased 17-OHCS output after burns in splanchnicectomized and partially sympathectomized animals with ablation of the central nervous system structures leaving behind the pituitary only has been observed in the present investigation. Any rise in 17-OHCS output in such animals after stress may not then be ascribable to the influence of the adrenomedullary hormones and neurohormones from the ablated sympathetic ganglia. It may be due to the action of burn toxins on the pituitary-adrenal-axis or lability of the same axis under these experimental conditions. Any fluctuation in pH or chemical alteration of the blood leads to increased activity of the axis. From observations mentioned previously we find that action of the toxins is manifested by a late rise in 17-OHCS output. However, in the group under discussion we find a very prompt response in absence of the nervous paths. This may be due to the more sensitive nature of the axis when the central nervous system structures are ablated.

### CONCLUSION

1. Burn trauma is a continued type of stress with increased adrenocortical activity.
2. In severely burned dogs patterns of increased adrenocortical activity in single or double extremity burn cannot be differentiated.
3. ACTH cannot stimulate the maximally stimulated adrenal cortex further.
4. Quick amputation of a burned extremity brings down the increased adrenocortical activity.
5. Presence of *wound hormone* in the burned area is suspected because of the late rise in adrenocortical activity from the following experiments :
  - (a) Subtotal isolation of the hind limb with absence of the neural link.
  - (b) Cross circulation experiments—dog B is connected to the hind limb of dog A by only vascular pathway.
  - (c) Limb grafting experiments.
6. That the presence of neural link is essential for the prompt adrenocortical response after burn is proved by the following experiments :
  - (a) Single and double extremity burn groups.
  - (b) Subtotal isolation of the hind limb with neurovascular link intact.
  - (c) Cross circulation experiments—dog A whose isolated limb is connected to the rest of the body only by neural link.
7. Neurectomy after burn does not bring down the already increased 17-OHCS output immediately to a basal level. The fall depends upon time interval at which the neurectomy is done after burn.
8. Splanchnicectomy and partial sympathectomy have no effect on the quick increased response of the adrenals after burns.



9. Prompt increased adrenocortical activity after ablation of central nervous system structures except the pituitary in splanchnicectomized and partially sympathectomized dogs after burn may be due to the action of the burn toxins on the isolated pituitary-adrenal-axis which becomes highly sensitive in such preparations.

TABLE 9

*Subtotal isolation of the limb (femoral artery, vein and sciatic nerve intact) and burn. Sciatic neurectomy performed 30 minutes after burn.*

<i>Dog Nos. Observation Nos.</i>	85	86	87	88	89	90	<i>Mean S.D. D.F.</i>
	<i>Adrenal venous 17-OHCS output (micrograms/minute)</i>						
1. During adrenal vein cannulation.	8.2	7.1	10.2	7.8	9.0	7.0	8.217 1.221 5
2. Forty eight hours after cannulation.	2.2	1.6	2.5	1.7	2.1	1.9	2.000 0.335 5
3. Thirty minutes after isolation of hind limb.	14.0	11.6	13.9	11.5	12.5	11.0	12.417 1.283 5
4. Sixty minutes after isolation	10.6	11.0	12.0	9.2	10.2	10.1	10.517 0.943 5
5. Thirty minutes after burn.	25.5	21.0	22.5	22.4	27.4	21.2	23.333 2.561 5
6. Thirty minutes after sciatic neurectomy.	18.6	19.2	16.3	17.2	21.3	16.0	18.100 2.008 5
7. Sixty minutes after sciatic neurectomy.	12.3	15.4	13.1	16.0	18.1	13.0	14.650 2.233 5
8. Three hours after sciatic neurectomy.	7.8	8.3	6.9	9.5	8.5	7.5	8.083 0.900 5
9. ACTH (subcutaneous injection into the burned limb).	12.0	14.7	11.7	14.5	16.0	13.0	13.650 1.691 5



TABLE 10

*Subtotal isolation of the limb (femoral artery, vein and sciatic nerve intact) and burn. Sciatic neurectomy performed 60 minutes after burn.*

Dog numbers Observation numbers.	91	92	93	94	95	96	Mean	S.D.	D.F.
	Adrenal venous 17-OHCS output (micrograms/minute)								
2. During adrenal vein cannulation ..	7.5	8.6	9.5	7.8	6.5	8.5	8.067	1.037	5
2. Forty-eight hours after cannulation ..	1.8	2.1	2.0	2.1	1.7	1.9	1.933	0.163	5
3. Thirty minutes after isolation of hind limb	13.0	12.6	11.9	12.0	11.2	13.0	12.283	0.711	5
5. Sixty minutes after isolation of hind limb	13.2	11.4	10.0	11.5	9.0	12.0	11.183	1.486	5
5. Thirty minutes after burn ..	27.7	26.5	22.0	24.7	22.0	26.3	24.867	2.417	5
6. Sixty minutes after burn ..	22.4	19.2	17.5	21.4	18.6	24.0	20.510	2.489	5
8. Thirty minutes after sciatic neurectomy	18.5	19.5	17.0	22.0	20.4	23.5	20.150	2.356	5
8. Sixty minutes after sciatic neurectomy ..	16.3	16.7	15.2	19.5	17.3	20.1	17.517	1.906	5
9. Three hours after sciatic neurectomy ..	13.6	14.1	12.6	15.1	12.9	18.6	14.483	2.205	5
10. ACTH (subcutaneous injection into the burned limb).	20.0	19.4	18.3	20.7	14.6	21.1	19.017	2.379	5



TABLE 11

*Subtotal isolation of the limb (femoral artery, vein and sciatic nerve intact) and burn. Sciatic neurectomy performed 3 hours after burn*

Dog numbers Observation Nos.	97	98	99	100	101	102	Mean	S.D.	D.F.
	Adrenal venous 17-OHCS output (micrograms/minute)								
2. During adrenal vein cannulation ..	9.2	10.0	11.5	9.0	8.1	10.2	9.667	1.172	5
2. Forty-eight hours after cannulation ..	2.2	1.8	1.7	1.8	2.0	2.5	2.000	0.303	5
3. Thirty minutes after isolation of hind limb	13.0	12.4	11.4	12.6	10.4	13.0	12.133	1.633	5
5. Sixty minutes after isolation of hind limb	11.6	10.7	11.0	10.5	12.1	13.2	11.517	1.014	5
5. Thirty minutes after burn ..	25.1	22.0	20.5	27.3	24.6	23.5	23.833	2.400	5
6. Sixty minutes after burn ..	21.2	20.5	18.3	22.4	18.4	19.1	20.000	1.637	5
7. Three hours after burn ..	18.4	16.2	15.7	19.1	17.2	16.2	17.133	1.362	5
8. Thirty minutes after sciatic neurectomy ..	17.5	16.6	16.2	19.6	18.1	17.6	17.600	1.202	5
9. Sixty minutes after sciatic neurectomy ..	13.1	14.0	15.6	16.5	13.5	15.3	14.667	1.334	5
10. Three hours after sciatic neurectomy ..	13.0	17.4	14.4	15.1	16.5	13.0	14.900	2.868	5
11. ACTH (subcutaneous injection into the burned limb).	19.0	20.3	21.5	19.3	18.7	20.2	19.833	1.038	5



TABLE 12

Cross-circulation experiments

Pair numbers	1		2		3		4		5		
	Dog numbers	103 B	104 A	105 B	106 A	107 B	108 A	109 B	110 A	111 B	112 A
Observation numbers				Adrenal venous 17-OHCS output (micrograms/minute)							
1. During adrenal vein cannulation		12.5	9.5	10.3	16.1	6.5	14.0	8.1	10.0	7.9	8.0
2. After establishment of cross-circulation and discontinuity of the hind limb of the recipient dog except the sciatic nerve.		8.6	11.2	8.0	12.5	7.5	16.0	11.1	13.0	6.0	9.5
3. Thirty minutes after burn of cross-circulated limb.		9.0	17.6	9.7	20.1	7.9	26.4	9.2	20.4	5.3	21.0
4. Sixty minutes after burn of cross-circulated limb		10.2	12.5	8.9	16.0	12.0	19.9	10.6	21.7	7.0	17.4
5. Two hours after burn of cross-circulated limb		15.5	18.7	12.8	18.1	18.1	22.6	14.0	17.5	12.1	15.8
6. Three hours after burn of cross-circulated limb		18.9	15.1	15.6	19.0	22.0	16.7	16.7	15.0	14.5	19.2
7. Four hours after burn of cross-circulated limb		20.1	14.0	16.7	16.3	21.0	13.4	18.5	16.1	16.1	17.6



TABLE 12

*Cross-circulation experiments—contd.*

Pair numbers	Dog numbers	Observations	6		7		8		Mean	
			113 B	114 A	115 B	116 A	117 B	118 A	B	A
Adrenal venous 17-OHCS output (micrograms/minute)										
1.	During adrenal vein cannulation	--	13.2	9.9	9.0	15.6	8.5	12.2	9.500	11.913
2.	After establishment of cross-circulation and discontinuity of the hind limb of the recipient dog except the sciatic nerve.		12.5	7.6	7.0	16.0	10.4	8.9	8.888	11.838
3.	Thirty minutes after burn of cross-circulated limb.		13.0	18.5	8.7	26.1	10.7	19.9	9.190	21.250
4.	Sixty minutes after burn of cross-circulated limb		14.5	20.0	6.9	23.1	13.0	18.2	10.388	18.600
5.	Two hours after burn of cross-circulated limb		18.9	23.0	11.5	21.3	18.2	16.9	15.138	19.238
6.	Three hours after burn of cross-circulated limb		21.2	18.4	14.0	15.8	19.5	16.0	17.800	16.900
7.	Four hours after burn of cross-circulated limb		19.7	15.2	17.0	14.6	21.0	13.8	18.763	15.125
			S. D.				D. F.			
			B		A		B		A	
1. During adrenal vein cannulation			2.333		3.033		7		7	
2. After establishment of cross-circulation and discontinuity of the hind limb of the recipient dog except the sciatic nerve.			2.234		3.137		7		7	
3. Thirty minutes after burn of cross-circulated limb.			2.208		3.270		7		7	
4. Sixty minutes after burn of cross-circulated limb			2.733		3.361		7		7	
5. Two hours after burn of cross-circulated limb			2.968		2.715		7		7	
6. Three hours after burn of cross-circulated limb			3.040		1.726		7		7	
7. Four hours after burn of cross-circulated limb			1.146		1.451		7		7	



TABLE 13

*Effect of burn of the grafted limb on adrenal venous 17-OHCS output in the recipient dogs*

Dog numbers Observation numbers.		119	120	121	122	123	124	Mean	S.D.	D.F.



TABLE 14

*Ablation of all central nervous system structures including the spinal cord leaving behind the pituitary only and burn*

Dog numbers Observation numbers.	125	126	127	128	129	130	Mean	S.D.	D.F.
	Adrenal venous 17-OHCS output (micrograms/minute)								
1. During adrenal vein cannulation	11.5	8.5	7.9	9.0	13.4	10.0	10.050	2.072	5
2. Forty-eight hours after cannulation	2.0	1.7	0.8	1.5	4.1	1.5	2.933	1.132	5
3. Thirty minutes after the preparation	1.2	0.9	0.7	1.9	2.8	1.0	2.417	0.794	5
4. Sixty minutes after the preparation	0.9	1.0	1.2	1.6	1.0	0.6	1.050	0.333	5
5. Interval 3 hours. Before burn	8.0	6.9	4.9	9.2	7.8	6.5	7.217	1.475	5
6. Thirty minutes after burn	10.5	8.9	5.7	12.5	9.2	7.0	8.967	2.426	5
7. Sixty minutes after burn	14.5	15.0	8.4	16.1	16.7	10.4	13.517	3.343	5
8. Two hours after burn	19.3	17.6	11.0	17.4	21.5	15.0	16.967	3.651	5
9. Three hours after burn	12.0	10.6	7.4	12.5	13.8	8.6	10.817	2.440	5
10. ACTH (I.V.)	16.7	19.3	14.2	18.0	19.0	15.3	17.083	2.053	5



TABLE 15

*Effect of burn on eplanchnicectomized and partially sympathectomized dogs*

Dog numbers Observation numbers.	131	132	133	134	135	136	Mean	S.D.	D.F.
	Adrenal venous 17-OHCS output (micrograms/minute)								
1. During adrenal vein cannulation	11.7	8.4	12.0	10.2	7.6	6.2	9.350	2.330	5
2. Forty-eight hours after cannulation	3.6	3.0	2.5	1.8	0.9	0.8	2.100	2.135	5
3. Thirty minutes after burn	29.9	27.5	28.0	25.6	23.0	24.1	26.350	2.588	5
4. Sixty minutes after burn	27.1	24.7	24.9	20.1	19.7	22.0	23.083	2.952	5
5. Three hours after burn	22.5	26.4	20.1	18.5	22.0	17.6	21.183	3.188	5
6. Twenty-four hours after burn	17.3	16.0	13.6	15.0	23.1	12.4	16.233	3.781	5
7. Forty-eight hours after burn	21.4	17.1	20.2	18.3	20.0	17.0	19.000	1.806	5
8. ACTH (subcutaneous injection into the burned limb).	26.0	27.3	32.0	28.5	31.6	29.0	29.067	2.361	5



STATISTICAL TABLE 1

*Values of 't' together with d. f. for statistical tests of significance for different types of "intra-group" paired comparisons regarding adrenal venous 17-OHCS output in dogs (effect of burn—single extremity on dogs).*

Observation No.	1 vs.	Observation No.	2	D.F.	"t"
				14	9.660***
"	2 vs.	"	3	14	—24.271***
"	2 vs.	"	4	14	—17.010***
"	2 vs.	"	5	14	—13.027***
"	2 vs.	"	6	14	—13.063***
"	2 vs.	"	7	14	—19.301***
"	7 vs.	"	8	14	—10.380***

STATISTICAL TABLE 2

*Values of 't' together with d.f. for statistical tests of significance for different types of "intra-group" paired comparisons regarding adrenal venous 17-OHCS output in dogs (burn-both hind limbs).*

Observation No.	1 vs.	Observation No.	2	D.F.	"t"
				4	5.479**
"	2 vs.	"	3	4	—26.384***
"	2 vs.	"	4	4	— 8.931***
"	2 vs.	"	5	4	—11.182***
"	2 vs.	"	6	3	— 9.460***
"	2 vs.	"	7	2	— 5.642*
"	7 vs.	"	8	2	— 4.000

STATISTICAL TABLE 3

*Values of 't' together with d.f. for statistical tests of significance for different types of "intra-group" paired comparisons regarding adrenal venous 17-OHCS output in dogs (amputation).*

Observation No.	1 vs.	Observation No.	2	D.F.	"t"
				14	15.014***
"	2 vs.	"	3	14	—14.151***
"	2 vs.	"	4	14	—18.960***
"	2 vs.	"	5	14	—12.983***
"	2 vs.	"	6	14	— 7.668***
"	2 vs.	"	7	14	— 2.972*
"	7 vs.	"	8	14	—17.014***



STATISTICAL TABLE 4

Values of 't' together with d.f. for statistical tests of significance for different types of "intra-group" paired comparisons regarding adrenal venous 17-OHCS output in dogs (amputation of the burned limb—amputation performed one hour after burn).

Observation No.	1	vs.	Observation No.	2	D.F.	"t"
	1	vs.		2	4	8.184**
"	2	vs.	"	3	4	-21.826***
"	2	vs.	"	4	4	-14.632***
"	4	vs.	"	5	4	-20.000***
"	4	vs.	"	6	4	2.852*
"	4	vs.	"	7	4	9.639***
"	4	vs.	"	8	4	7.987**
"	4	vs.	"	9	4	8.629***
"	9	vs.	"	10	4	-10.503***

STATISTICAL TABLE 5

Values of 't' together with d.f. for statistical tests of significance for different types of "intra-group" paired comparisons regarding adrenal venous 17-OHCS output in dogs (amputation of the burned limb—amputation performed two hours after burn).

Observation No.	1	vs.	Observation No.	2	D.F.	"t"
	1	vs.		2	5	15.960***
"	2	vs.	"	3	5	-31.587***
"	2	vs.	"	4	5	-12.303***
"	2	vs.	"	5	5	-13.586***
"	5	vs.	"	6	5	-4.135**
"	5	vs.	"	7	5	-0.661
"	5	vs.	"	8	5	4.900**
"	5	vs.	"	9	5	4.733**
"	5	vs.	"	10	5	9.648***
"	10	vs.	"	11	5	-11.765***



STATISTICAL TABLE 6

Values of 't' together with d.f. for statistical tests of significance for different types of "intra-group" paired comparisons regarding adrenal venous 17-OHCS output in dogs (amputation of the burned limb—amputation performed three hours after burn).

Observation No.	1 vs.	Observation No.	2	D.F.	"t"
				4	11.649***
"	2 vs.	"	3	4	-15.961***
"	2 vs.	"	4	4	-8.484**
"	2 vs.	"	5	4	-11.315***
"	2 vs.	"	6	4	-9.177***
"	6 vs.	"	7	4	-38.434***
"	6 vs.	"	8	4	-7.083**
"	6 vs.	"	9	4	-0.334
"	6 vs.	"	10	4	5.527**
"	6 vs.	"	11	4	10.139***
"	11 vs.	"	12	4	-19.415***

STATISTICAL TABLE 7

Values of 't' together with d.f. for statistical tests of significance for different types of "intra-group" paired comparisons regarding adrenal venous 17-OHCS output in dogs (subtotal isolation of the limb—femoral artery, vein and sciatic nerve intact and burn).

Observation No.	1 vs.	Observation No.	2	D.F.	"t"
				4	8.272**
"	2 vs.	"	3	4	-8.220**
"	2 vs.	"	4	4	-11.354***
"	4 vs.	"	5	4	-15.175***
"	4 vs.	"	6	4	-5.131**
"	4 vs.	"	7	4	-3.205*
"	7 vs.	"	8	4	-6.070**



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STATISTICAL TABLE 8

Values of 't' together with d.f. for statistical tests of significance for different types of "intra-group" paired comparisons regarding adrenal venous 17-OHCS output in dogs (subtotal isolation of the limb—femoral artery and vein intact and burn. Sciatic neurectomy performed during isolation of the limb).

Observation No.	1 vs.	Observation No.	2	D.F.	"t"
				7	11.930***
"	2 vs.	"	3	7	—14.079***
"	2 vs.	"	4	7	—13.094**
"	4 vs.	"	5	7	0.880
"	4 vs.	"	6	7	— 2.280
"	4 vs.	"	7	7	—10.693***
"	7 vs.	"	8	7	—10.820***

STATISTICAL TABLE 9

Values of 't' together with d.f. for statistical tests of significance for different types of "intra-group" paired comparisons regarding adrenal venous 17-OHCS output in dogs (subtotal isolation of the limb-femoral artery, vein and sciatic nerve intact and burn. Sciatic neurectomy performed 30 minutes after burn).

Observation No.	1 vs.	Observation No.	2	D.F.	"t"
				5	16.063***
"	2 vs.	"	3	5	—21.303***
"	2 vs.	"	4	5	—26.603***
"	4 vs.	"	5	5	—11.107***
"	5 vs.	"	6	5	7.110***
"	5 vs.	"	7	5	7.901***
"	5 vs.	"	8	5	14.346***
"	8 vs.	"	9	5	—11.381***



STATISTICAL TABLE 10

Values of 't' together with d.f. for statistical tests of significance for different types of "intra-group" paired comparisons regarding adrenal venous 17-OHCS output in dogs (subtotal isolation of the limb-femoral artery, vein and sciatic nerve intact and burn. Sciatic neurectomy performed 60 minutes after burn).

Observation No.	1 vs.	Observation No.	2	D.F.	"t"
				5	16.055***
"	2 vs.	"	3	5	-36.316***
"	2 vs.	"	4	5	-15.488***
"	4 vs.	"	5	5	-29.175***
"	4 vs.	"	6	5	-14.956***
"	6 vs.	"	7	5	0.466
"	6 vs.	"	8	5	4.208**
"	6 vs.	"	9	5	10.243***
"	9 vs.	"	10	5	-5.738**

STATISTICAL TABLE 11

Values of 't' together with d.f. for statistical tests of significance for different types of "intra-group" paired comparisons regarding adrenal venous 17-OHCS output in dogs (subtotal isolation of the limb-femoral artery, vein and sciatic nerve intact and burn. Sciatic neurectomy performed 3 hours after burn).

Observation No.	1 vs.	Observation No.	2	D.F.	"t"
				5	14.887***
"	2 vs.	"	3	5	-26.388***
"	2 vs.	"	4	5	-30.899***
"	4 vs.	"	5	5	-11.479***
"	4 vs.	"	6	5	-8.930***
"	4 vs.	"	7	5	-7.201***
"	7 vs.	"	8	5	-1.492
"	7 vs.	"	9	5	-3.196*
"	7 vs.	"	10	5	2.267
"	10 vs.	"	11	5	-5.618**



STATISTICAL TABLE 12

Values of 't' together with d.f. for statistical tests of significance for different types of "intra-group" paired comparisons regarding adrenal venous 17-OHCS output in dogs (cross-circulation experiments).

			Donor dogs		Recipient dogs	
			D.F.	"t"	D.F.	"t"
Observation No. 1	vs.	Observation No. 2	7	0.732	7	0.082
" 2	vs.	" 3	7	— 0.716	7	—13.563***
" 2	vs.	" 4	7	— 2.673*	7	— 5.275**
" 2	vs.	" 5	7	— 7.567***	7	— 6.106***
" 2	vs.	" 6	7	— 9.452***	7	— 3.487*
" 2	vs.	" 7	7	—13.273***	7	— 2.441*

STATISTICAL TABLE 13

Values of 't' together with d.f. for statistical tests of significance for different types of "intra-group" paired comparisons regarding adrenal venous 17-OHCS output in dogs (effect of burn of the grafted limb on adrenal venous 17-OHCS output in the recipient dogs).

			D.F.	"t"
Observation No. 1	vs.	Observation No. 2	5	7.420***
" 2	vs.	" 3	5	— 5.569**
" 3	vs.	" 4	5	— 0.500
" 3	vs.	" 5	5	— 3.276*
" 3	vs.	" 6	5	—13.077***
" 3	vs.	" 7	5	— 6.864***
" 7	vs.	" 8	5	— 6.220**



STATISTICAL TABLE 14

*Values of 't' together with d.f. for statistical tests of significance for different types of "intra-group" paired comparisons regarding adrenal venous 17-OHCS output in dogs (ablation of all central nervous system structures including the spinal cord—leaving behind the pituitary only and burn).*

Observation No.	1	vs.	Observation No.	2	D.F.	"t"
	1	vs.	2	5		17.307***
"	2	vs.	"	3	5	2.117
"	2	vs.	"	4	5	1.752
"	2	vs.	"	5	5	-9.000***
"	5	vs.	"	6	5	-4.042**
"	5	vs.	"	7	5	-7.047***
"	5	vs.	"	8	5	-8.880***
"	5	vs.	"	9	5	-6.406**
"	9	vs.	"	10	5	-3.264*

STATISTICAL TABLE 15

*Values of 't' together with d.f. for statistical tests of significance for different types of "intra-group" paired comparisons regarding adrenal venous 17-OHCS output in dogs (burn on splanchnicectomized and partially sympathectomized dogs).*

Observation No.	1	vs.	Observation No.	2	D.F.	"t"
	1	vs.	2	5		10.522***
"	2	vs.	"	3	5	-39.050***
"	2	vs.	"	4	5	-25.159***
"	2	vs.	"	5	5	-17.459***
"	2	vs.	"	6	5	-8.493***
"	2	vs.	"	7	5	-24.074***
"	7	vs.	"	8	5	-8.831***

\* = Significant at 5% level

\*\* = Significant at 1% level

\*\*\* = Significant at 0.1% level or more stringent level.



STATISTICAL TABLE 16

Showing d.f. and values of 't' s' and their significance for different types of "inter-group" comparisons.

Table 1 vs. Table 2				D.F.	"t"
Observation No.	3 vs.	Observation No.	3	18	-3.863**
"	4 vs.	"	4	18	-2.048
"	5 vs.	"	5	18	-1.990
"	6 vs.	"	9	14	-2.776*

STATISTICAL TABLE 17

Showing d.f. and values of 't' s' and their significance for different types of "inter-group" comparisons.

Table 1 vs. Table 13				D.F.	"t"
Observation No.	3 vs.	Observation No.	4	19	6.100***
"	4 vs.	"	5	19	3.772**
"	5 vs.	"	6	19	0.556

STATISTICAL TABLE 18

Showing d.f. and values of 't' s' and their significance for different types of "inter-group" comparisons.

Table 7 vs. Table 8				D.F.	"t"
Observation No.	3 vs.	Observation No.	3	11	2.287*
"	4 vs.	"	4	11	1.450
"	5 vs.	"	5	11	8.481***
"	6 vs.	"	6	11	3.115**
"	7 vs.	"	7	11	0.324
"	8 vs.	"	8	11	0.042

\* = Significant at 5% level

\*\* = Significant at 1% level

\*\*\* = Significant at 0.1% level or more stringent level.





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## CHAPTER—2

### EFFECT OF ANAESTHESIA ON ADRENALS (1969)

#### INTRODUCTION

Scaglione(1915) found the lipid content of the adrenal cortex to diminish after chloroform anaesthesia in the guinea pig. Stress changes in adrenal and its enlargement can be found after barbiturates (Chanutin and Ludewig, 1946 ; and Ludewig and Chanutin, 1947).

Marked fall in adrenal ascorbic acid was noted after ether (Bowman and Muntwyler, 1936-1937 ; Lauber *et al.*, 1937 ; and Mitamura, 1960), urethane (Vogt, 1955 ; and Mitamura, 1960) and morphine (Nasmyth, 1954 ; Briggs and Munson, 1955 ; George and Way, 1955 ; and Mitamura (1960). Lauber *et al.* (1937) and Mitamura (1960) noted very slight depletion of adrenal ascorbic acid after sodium hexobarbital anaesthesia. Van Peenen and Way (1957) did not find any effect on the adrenal ascorbic acid content of the rat after sodium pentobarbital anaesthesia.

Suzuki *et al.* (1959a) and Suzuki *et al.* (1959b) noted the effects of ether or morphine on adrenal venous 17-OHCS content in dogs. Harwood and Mason (1957) observed depression in the peripheral blood 17-OHCS level after sodium pentobarbital anaesthesia in monkeys. Walker *et al.* (1959) did not find any significant change in corticosteroid secretion rate after thiopental injection in the dog. Suzuki *et al.* (1962) observed decrease in the secretion rate of 17-OHCS after barbiturate injection in the dog.

Moore (1955) said that after ether anaesthesia there was rise in adrenal venous blood corticoid level. Sydnor and Sayers (1954) studied blood and pituitary ACTH in intact and adrenalectomized rats subjected to ether anaesthesia and other stresses of the type of scald and ex-sanguination. Sayers and Burks (1955) observed blood ACTH changes during ether anaesthesia in adrenalectomized rats. There was fall in the level of circulating ACTH gradually. Excitation of the central nervous system during starting of ether anaesthesia accelerates the release of ACTH. With the progress of anaesthesia the CNS excitability is reduced and so ACTH discharge is reduced. The other explanation of the finding is that the hypothalamic neurohumoral secretory mechanism is exhausted after prolonged excitation.

Sandberg *et al.* (1954) noted rise of plasma 17-OHCS level in subjects under general anaesthesia even before surgery.

Royce and Sayers (1958) observed biphasic response after ether. It first excited and then inhibited ACTH release. Pentobarbital had a depressant action. Blocking of the excitatory phase of ether can be done by decerebration and the destruction of the median eminence. The inhibitory action of ether and pentobarbital on ACTH release is due to the involvement of the multisynaptic conduction system (the brainstem reti-



cular system). Sayers (1957) observed that the section through the brain-stem at the upper level of pons abolished the excitatory phase of the pituitary response to ether.

Anaesthetic action of morphine on hypothalamus was studied by Briggs and Munson (1954). Morphine leads to depletion of adrenal ascorbic acid in rats, but stressor action was abolished when the animals were first of all anaesthetised with pentobarbital sodium (40 mg./kg.) ten minutes before the injection of morphine. Morphine then could block the discharge of ACTH after histamine, vasopressin or laparotomy. Depletion of adrenal ascorbic acid could not be blocked by morphine in hypophysectomised rats. Unanaesthetised rats accustomed to morphine by daily injection for four days no longer responded to morphine by increased ACTH secretion. Action of histamine is blocked in such rats.

Cronheim and Hyder (1954) observed that deep anaesthesia with pentobarbital blocked the adrenal ascorbic acid depletion after subcutaneous injection of salicylates.

### MATERIALS AND METHODS

Male dogs of 10 to 15 kg. in weight have been used in this experiment. Cannulation of the right lumbo-adrenal vein for the collection of adrenal venous blood was done after the method of Hume and Nelson (1955). Adrenal venous blood samples have been collected in heparinised tubes. The dogs were not heparinised. Blood was centrifuged immediately and plasma was preserved in the cold for the determination of 17-hydroxycorticosteroids after the method of Silber and Porter (1954). The animals were anaesthetised with either ether or pentobarbital sodium (nembutal—Abbott laboratories) for cannulation of adrenal vein according to the particular type of anaesthetics used afterwards *i.e.*, when we used ether subsequently, the same anaesthetic was used during cannulation. The same method holds true also for nembutal anaesthesia. Rest for forty eight hours was allowed to the animals. The pre-anaesthetic resting blood sample was drawn and the animal was subsequently anaesthetised with either ether or nembutal. Nembutal (5% solution—30 mg./kg.) was injected into the saphenous vein.

With ether anaesthesia there are two observations, one at 30 minutes and the other at 60 minutes. After nembutal anaesthesia the adrenal venous blood was collected at 15, 30, 45, 60 and 120 minutes.

### RESULTS

*Ether Anaesthesia* : (Table No. 1 and Statistical Table No. 1 : Fig. No. 1).

Thirty minutes after ether anaesthesia the adrenal venous 17-OHCS output was high and the difference with the basal level was highly significant at 0.1% level. At sixty minutes the corticosteroid output was further elevated (highly significant at 0.1% level) when this was compared to the basal corticosteroid output. The difference between the observations made at 30 and 60 minutes was also significant at 1% level.



*Nembutal anaesthesia* : (Table No. 2 and Statistical Table No. 2 ; Fig. No. 2).

When the adrenal venous 17-OHCS output at 15, 30, 45, 60 and 120 minutes were compared to the basal 17-OHCS output, the differences were found to be highly significant at 0.1% level. Comparisons of the output between 15 and 30 minutes, 30 and 45 minutes, 45 and 60 minutes, and 60 and 120 minutes were found to be nonsignificant. In all these animals the depth of anaesthesia persisted for more than 120 minutes.

### DISCUSSION

After ether anaesthesia there is an increase of ACTH secretion (Hammond *et al.*, 1958 ; Hume, 1957 ; Klein *et al.*, 1955 ; Suzuki *et al.*, 1959 ; Vandam and Moore, 1960 ; Virtue *et al.*, 1957 and Roy, 1959). Similar results have been noted here also and even at 60 minutes there is a tendency for further rise of the corticosteroid output over that noted at 30 minutes. This experiment has been repeated here only to show the stimulatory action of ether against the depressive action of pentobarbital sodium. It is our experience that surgery carried out under ether anaesthesia leads to further rise of corticosteroid secretion both in the man and in the dog over that noted after anaesthesia alone in the same subject or animal. This may probably be due to depression of the hepatic and renal function and thereby unconjugated 17-OHCS is slowly removed from the blood and thus its rise in the blood is noted.

Kaada *et al.* (1959) noted that without much excitement during induction, pentobarbital anaesthesia had no effect on adrenocortical secretion in the dog. Royce and Sayers (1958) observed decrease of ACTH secretion after this anaesthesia in rats and Sikar *et al.* (1958) noted slight lowering of plasma unconjugated 17-OHCS after subanaesthetic doses of pentobarbital in man. Suzuki *et al.* (1962) observed lowering of the secretion rate of 17-OHCS after barbiturate injection when simultaneous abdominal surgery was avoided. Our present investigation confirms this. Van Brunt and Ganong (1963) however, noted that in the dog 17-OHCS output after surgical interference was just as high in pentobarbital anaesthetised dogs as in those with ether anaesthesia. They further mention that the difference between the two types of anaesthesia is that, 17-OHCS output remains high after operation as long as the effect of ether anaesthesia is present but a variable decline is noted after surgery under pentobarbital anaesthesia.

*Site of action of pentobarbital sodium* :—Our present investigation does not throw any light on this particular aspect. However, that it is not at the pituitary-adrenal level is proved by the experiments of Richard and Egdahl (1956) and Roy (1959). The previous authors showed that sodium pentobarbital anaesthesia in intact and in hypophysectomized dogs did not lead to any change in adrenal 17-OHCS secretory response after ACTH. Roy (1959) studied the percentage rise in 17-OHCS output in adrenal venous blood of the assay dogs after intracarotid injection of hypothalamic extracts from normal, stressed, hypophysectomized and hypophysectomized-stressed dogs. Pentobarbital sodium anaesthesia in



the assay dogs could not prevent the increased adrenocortical response, proving thereby that the CRF action of the hypothalamic extract on the pituitary was not blocked. Hypophysectomy in the assay animals blocked the response and presence of hypothalamus was not essential for the mediation of the response. Egdahl (1961) failed to find post-anaesthetic depression of high resting corticosteroid levels in dogs after removal of brain and this is an evidence which speaks against a direct action of nembutal on the adrenal. Similar finding has been noted here also and the results have been put separately (Roy, 1966). The inhibitory action of pentobarbital sodium is at the level of the central nervous system. French *et al.* (1953) said that the reticular formation is the main site of action of nembutal.

### CONCLUSION

Ether anaesthesia in dogs without surgery leads to increased adrenal venous 17-OHCS output. Intravenous injection of pentobarbital sodium lowers the 17-OHCS output in absence of surgery.

TABLE 1  
*Effect of Ether Anaesthesia*

Dog No.	Weight in Kg.	Adrenal venous 17-OHCS output (microgram/minute)		
		Before anaesthesia	After anaesthesia and without surgery	
			30 min.	60 min.
1	15	1.5	8.4	9.8
2	12	1.8	10.1	13.4
3	10.5	2.6	9.5	14.5
4	11.5	2.8	13.6	11.6
5	14	1.9	12.0	15.2
6	11	3.5	9.0	11.6
7	13	2.2	8.6	12.0
8	10	2.7	11.9	14.0
9	12	2.1	14.0	18.0
10	14	2.0	9.0	10.5
Mean		2.310	10.610	13.060
S. D.		0.599	2.098	2.463
D. F.		9	9	9



# EFFECT OF ANAESTHESIA ON ADRENALS

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STATISTICAL TABLE I

Values of 't' together with d.f. for statistical tests of significance for different types of "intra-group" paired comparisons regarding adrenal venous 17-OHCS output in dogs (ether anaesthesia).

Observation No 1 vs		Observation No. 2		D.F.	"t"
				9	—12.401***
g <sub>1</sub>	1 vs	"	3	9	—113.372***
"	2 vs.	"	3	9	— 4.044***

TABLE II

*Effect of Nembutal Anaesthesia*

Dog No.	Weight in kg.	Before injection of nembutal	Adrenal venous 17-OHCS output (microgram/minute)				
			15 min.	30 min.	45 min.	60 min.	120 min.
11	12	1.9	1.2	1.0	0.8	1.1	0.9
12	10	2.7	1.4	1.3	1.0	1.2	1.5
13	15	2.0	1.2	0.9	1.0	1.1	1.0
14	13	3.1	1.5	1.9	1.3	1.5	1.3
15	11	2.5	1.0	1.2	1.1	1.5	1.0
16	12	1.6	0.8	1.1	1.0	0.9	1.2
17	12.5	2.2	1.3	1.5	1.5	1.0	1.1
18	10	1.5	0.9	1.0	1.0	0.9	1.0
19	13.5	1.4	1.1	1.0	0.9	0.8	1.0
20	14	2.1	1.2	1.0	1.1	1.3	1.1
Mean		2.100	1.160	1.190	1.070	1.130	1.110
S. D.		0.546	0.217	0.307	0.200	0.245	0.179
D. F.		9	9	9	9	9	9



STATISTICAL TABLE II

Values of "t" together with d.f. for statistical tests of significance for different types of "intragroup" paired comparisons regarding adrenal venous 17-OHCS output in dogs (nembutal anaesthesia).

Observation No. 1 vs.	Observation No. 2	D.F.	"t"
1 vs.	3	9	7.287***
1 vs.	4	9	7.913***
1 vs.	5	9	6.913***
1 vs.	6	9	8.584***
2 vs.	3	9	6.781***
3 vs.	4	9	-0.395
4 vs.	5	9	1.791
5 vs.	6	9	-0.723
			0.241

S.D. —Standard deviation of observations.

D.F. —Degrees of freedom.

\* —Significant at 5% level.

\*\* —Significant at 1% level.

\*\*\* —Significant at 0.1% level or more stringent level.

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## CHAPTER 3

OBSERVATIONS AND CRITICAL  
REVIEWS ON CORTICOTROPHIN RELEASING  
FACTOR ( C R F ) AND CORTICOTROPHIN  
INFLUENCING FACTOR ( C I F )  
( 1965 )



*The effect of the hypothalamic and neurohypophyseal corticotropin releasing factor (CRF), and corticotropin influencing factor (CIF) on the pituitary-adrenocortical activity.*

Authors and Year	Source and method of preparation of the extract.	Route of administration.	Dose and type of receptor animal.	Response
1	2	3	4	5
Hume, D. M. (1949)	Extract of hypothalamus.		Normal animals and animals with hypothalamic lesions.	Eosinopenia
Hume, D. M., and Wittenstein, G. J. (1950).	Extracts from beef hypothalamus, cerebral cortex, and cerebellum.	Injected	Dogs with hypothalamic lesions showing absence or subnormal responses to two or more of the various stressing agents.	Release of ACTH (eosinopenia). Some specific substance is produced in the hypothalamus which is responsible for the response.
Hellerstein, S., and others (1952).	Hypothalamic, medulla oblongata and cerebral cortex extracts were suspensions in 5% glucose of substances derived by double acetone extraction of the respective beef tissues (1.5 vols. of acetone for first extraction and 1.0 vol. for second extraction).	Subcutaneous tissue of the dorsal region.	Infant rats six days after birth.—Hy. Ext. in 5% glucose—0.12 ml. every 24 hours—medullary ext. in 5% glucose—same dose—Brain cortex ext. in 5% glucose—same dose—ACTHAR-C—same dose.	Infant albino rats receiving daily injection of an extract of beef hypothalamus shows leucopenia as compared with litter mates having injection of extract of beef medulla oblongata. This leucopenia is a manifestation of depression of both polymorphonuclear and mononuclear cells. These animals develop a relative eosinophilia during leucopenia. The leucopenic effects of ACTH and hypothalamic extract have been compared.



Authors and Year	Source and method of preparation of the extract.	Route of administration.	Dose and type of receptor animal.	Response
1		3	4	5
Slasber, M. A., and Roberts, S. (1954).	<p>(a) Aqueous extract—Brain homogenized at 0° with 100 ml. water at pH 7.2, dialyzed 2 days at 4°C. Lyophilized. Non-dialysable protein fraction and dialysate Aqueous non-protein fraction.</p> <p>(b) Lipide extract (all procedures under N<sub>2</sub>). Brain extracted with 95% ethanol and ethyl ether, extracts combined and evaporated—resolved with petroleum ether; filtered. Combined lipids evaporated, saponified with 1N alc. KOH and extracted with petroleum ether. The ether extract washed with acid and evaporated. This gives rise to non-saponifiable lipide fraction. The aqueous extract acidified and extracted with petroleum ether. The residue is discarded and the extract evaporated (Saponifiable lipide fraction)</p>	Intra-peritoneal injection.	Protein fraction—dissolved in saline. Aqueous non-protein fraction—dissolved in saline. Both lipide fractions dissolved in sesame oil—0.1 ml. of each injected. Aqueous non-protein fraction of anterior hypothalamus and posterior hypothalamus each 0.08 mg/100 gm. body weight. Protein fraction of A. Hyp. and P. Hyp. = 5.0 mg./100 gm. body weight. The receptor animal is intact rat and hypophysectomized rats.	<p><i>Adrenal ascorbic acid depletion method and eosinopenia.</i></p> <p>Of the two purified extracts from bovine hypothalamus the first factor was a water soluble, non-protein substance which could also be extracted from all parts of the brain. The second factor was a lipid or lipoprotein and this was present only in the extracts of the posterior hypothalamus. This could activate only in the presence of the pituitary.</p> <p>"This lipoidal substance may represent in crude form the natural neurohumor presumed involved in the stress-induced release of ACTH by the adeno-hypophysis."</p>
Spigoion, G. (1955)	Lipid fraction of the diencephalic region.			Hyperplasia of the fascicular zone of the suprarenal with increased alkaline phosphatase.
Safran, M., and Schally, A. V. (1955).	Release of ACTH by rat anterior pituitary tissue <i>in vitro</i> was used for detection of factors that could stimulate ACTH release.—Hypothalamic tissue was dissected from the base of the brain and was cut into approximately 1 mm. cubes. Hypothalamus (rat) included		Posterior pituitary tissue in the medium = about 2 mgm. Brain cortex or hypothalamus = 15 mgm.	<p>(a) Epinephrine or arterenol added had no effect.</p> <p>(b) No effect by hypothalamic tissue.</p> <p>(c) Hypothalamic tissue and epinephrine or arterenol give rise to increased release of ACTH to about threefold.</p>



Authors and Year	Source and method of preparation of the extract.	Route of administration.	Dose and type of receptor animal.	Response
1	<p>median eminence. Brain cortex and liver tissue cubes of similar size were also used.</p>	3	4	5
<p>(d) Brain cortex could replace hypothalamus.</p> <p>(e) Greatest stimulation of ACTH release occurred with posterior pituitary tissue and arterenol. Arterenol may be replaced by hypothalamus or sphingosine, but not by dopamine which has got same structure as arterenol.</p>				
<p>Saffran, M., Schally, A. V., and Benfey, B. G. (1955).</p>	<p>ACTH release was measured by <i>in vitro</i> method. Neurohypophysis of 6 rats (total tissue weight, 4.5 mg.) in 75 microliter was ground with sand and glass rod. 30 microliter of the extract were incubated with anterior pituitary tissue &amp; arterenol for one hour. Posterior pituitary hormones from beef were further purified by paper chromatography. The area of greatest CRF activity was above that of vasopressin and clearly separate from it.</p>			<p>Posterior pituitary extracts contain a CRF.</p> <p>Vasopressin contains CRF as an impurity. CRF can be separated from Stehle's vasopressin by paper chromatography and is distinct from vasopressin and oxytocin. The activity of CRF is increased by arterenol (noradrenaline).</p>
<p>Curri, S. B., and Fedeli, S. (1955).</p>	<p>Lipid fraction of the diencephalic region.</p>			<p>Hyperplasia of the fascicular zone of the suprarenal with increased alkaline phosphatase.</p>
<p>Fedeli, S. (1956)</p>	<p>Lipid fraction of the diencephalic region.</p>			<p>Fall in adrenal cholesterol.</p> <p>Fall in circulating eosinophil.</p>





Authors and Year	Source and method of preparation of the extract.	Route of administration.	Dose and type of receptor animal.	Response
1	2	3	4	5
Schapiro, S., Marmorston, J., and Sobel, H. (1956).	Brain blood of stressed hypophysectomized rats and unstressed animals (posterior facial vein blood).	Injected through carotid cannula.	Hypothalamic lesioned rats. Hypophysectomized and adrenalectomized rats.	Marked eosinopenia occurred when brain blood from stressed hypophysectomized rats was injected into rats with hypothalamic lesions. The eosinopenia did not occur when brain blood from unstressed animals was injected into lesioned animals. Eosinopenia depends upon intact pituitary-adrenal axis.
Guillemin, R., Hearn, W. R., Cheek, W. R., and Housholder, D. E. (1956).	Extraction method same as after Guillemin <i>et al.</i> (1957).			
Porter, J. C., and Rumsfeld, H. W. (Jr.) (1956b).	The ACTH releasing activity in the portal vessel plasma was present in a globulin subfraction.	Intravenous injection.	Hydrocortisone—Inhibited intact rats.	ACTH release
Porter, J. C., and Rumsfeld, H. W. (Jr.) (1956a).	Blood was collected from the sella of dogs after hypophysectomy. The plasma was separated and stored in a frozen state. Lyophilized plasma was used. Purification was done by dialysis. The active substance in portal plasma is non-dialysable and following low temperature alcohol fractionation of the plasma proteins is found to be present in a single fraction. Further purification of this sub-fraction from 10 ml. of original plasma gave a fraction which contained 1.3 mg. of protein.	Intravenous injection via the tail vein.	Dose—2 injections of 2 ml., per injection. Receptor animal: (a) hypophysectomized rats. (b) hydrocortisone inhibited, intact rats.	Adrenal ascorbic acid depletion in hydrocortisone—inhibited, intact rats after injection of lyophilized portal vessel plasma. Pituitary is required for the response. This caused a mean change in adrenal ascorbic acid concentration of—77 mg./100 gm. adrenal weight. This is (a) non-dialysable and (b) not ACTH. Its activity is not due to epinephrine, norepinephrine or histamine.



Authors and Year	Source and method of preparation of the extract.	Route of administration.	Dose and type of receptor animal.	Response
1	2	3	4	5
Porter, J. C., and Jones, J. C. (1956).	Blood collected from the sella of dogs after hypophysectomy.	Intra-venous injection via the tail vein.	Receptor animals are hypophysectomized rats and hydrocortisone-inhibited, intact rats.  Dose : 2 ml., of plasma. In experiments where a total of 4 ml., per rat was used, the second injection of 2 ml. was given 30 minutes after the first.	Adrenal ascorbic acid depletion method. Blood from the hypophysectomized vessels contains a factor/s which accelerates the release of ACTH from the anterior lobe.

Guillemin, R. (1956).

Hypothalamic or some other control tissues (spleen, liver, brain cortex) were cultured with pituitary tissues—(tissue cultures). Aqueous homogenates (and saline extracts of posterior or anterior hypothalamus of bovine origin were added to the pituitary in organ culture. Homogenates of brain cortex (ox) were used as control. ACTH activity in the various fluids was measured in qualitative manner in hypophysectomized rats by adrenal ascorbic acid depletion method.

ACTH activity is never found after more than four days of *in vitro* life. When fragments of posterior hypothalamus or of median eminence are added to the culture medium of the pituitary tissues from fifteen to nineteen days or twenty two to twenty six days, ACTH activity was found again. No ACTH activity in the culture—fluids of hypothalamic tissues was noted.

No ACTH was released when brain cortex, spleen, liver, or anterior hypothalamus were added to the anterior pituitary.

In organ cultures of whole anterior pituitary *in vitro* there is no release of ACTH after more than two to four days of culture. The secretion of ACTH can be in-





Authors and Year	Source and method of preparation of the extract.	Route of administration.	Dose and type of receptor animal.	Response
1	2	3	4	5

creased and maintained up to eight days when incubated with homogenates of bovine posterior or anterior hypothalamus.

The substance/s of hypothalamic origin is not epinephrine, nor-epinephrine, histamine, acetylcholine, 5-hydroxytryptamine, oxytocin, or vasopressin.

Adrenal ascorbic acid depletion method. After the injection of the extracts there was a fall in adrenal ascorbic acid

in normal rats and no change in hypophysectomized rats. This proves that pituitary is essential for the response. The effect of the extract was found in nembutalized rats but not in nembutalized rats treated also with chlorpromazine. The effect of the extract was present in the group (e) of the receptor animals. This may mean that neither sympathetic, parasympathic, comimetic, ganglion-transmission nor histamine release is necessary for the activity of the extract. The author concludes that the effect of the extract depends upon the function of the hypothalamic centers.

0.2 ml. (10 mg. extract) per 100 gm. body weight.

Tested in:  
(a) normal rats,  
(b) hypophysectomized rats,  
(c) nembutalized rats,  
(d) rats treated with nembutal and chlorpromazine,  
(e) nembutalized rats treated with di-hydroergotamine, atropine sulphate, neostergan or hexamethonium chloride.

Intra-peritoneal injection.

Bovine posterior hypothalamus. Non-saponifiable lipid extract was prepared after the method of Slusher and Roberts (1954). This was dissolved in sesame oil before injection.

4. Wied, D. (1957)

Bovine posterior hypothalamus. Non-saponifiable lipid extract was prepared after the method of Slusher and Roberts (1954). This was dissolved in sesame oil before injection.



Authors and Year	Source and method of preparation of the extract.	Route of administration.	Dose and type of receptor animal.	Response
1	2	3	4	5
Guillemin, R., Hearn, W. R., Cheek, W. R., and Housholder, D. E. (1957).	<p>Hypothalamic tissue, brain cortex and cerebellum from pork and beef.</p> <p>Extraction process was started with a minimum of 50 gm. of hypothalamic or other brain tissues.</p> <p>(1) The tissues were treated after the method of <i>Kamm et al.</i> (1928). The final product was dealt with as in 2b.</p> <p>(2a) Fresh tissues extracted 5 times in 10 parts of 0.5% acetic acid for 15 min. at room temperature. Pooled extracts frozen overnight. Thawed and centrifuged for 30 min. the next morning. Supernatant was lyophilized, defatted with petroleum ether or <math>CCl_4</math> and next dealt with as in 2b.</p> <p>(2b) Extraction with 90% methanol. Centrifuged. The supernatant was poured off and the methanol evaporated in <i>vacuo</i> at 35° to 40°C in a water bath. This methanolic extract has got the material which stimulates ACTH release.</p> <p>(2c) The extract weighed and taken up in water for ascending chromatography for 12-15 hours at room temperature. Solvent system = Acetone 60 parts, diethylene glycol 10 parts, 0.5% aqueous 30 parts. Side strips of each sheet were developed with ninhydrin in butanol and the fraction eluted with 0.25% aq. acetic acid. Eluates lyophilized and excess an-</p>			<p>Stimulation of ACTH release and assessments of ACTH release were done by <i>in vitro</i> pituitary.</p> <p>Release of ACTH from rat pituitary tissue <i>in vitro</i> occurred by a substance isolated from the hypothalamus and the posterior pituitary. The purified material appears to be different from vasopressin, oxytocin, ACTH, histamine, acetylcholine, adrenalin, noradrenalin, and 5-hydroxytryptamine. The activity is located in a common fraction (fraction D.).</p>





Authors and Year	Source and method of preparation of the extract.	Route of administration.	Dose and type of receptor animal.	Response
1	2	3	4	5
	<p>hydrous ethyl acetate added to the residue to remove nonvolatile diethylene glycol. The material precipitated by centrifugation was taken. The ethyl acetate decanted and the precipitate taken in a few ml. of water with one drop of glacial acetic acid and lyophilized.</p>			
Mc Cann, S. M. (1957).	<p>Ascending chromatography was done with pitressin for 18 hours in the following solvent system. 2-2 oxydiethanol, 10 ml., Acetone, 60 ml., 0.5% aqueous urea 30 ml. The adjacent strip was developed with 0.25% ninhydrin in acetone by heating at 80°C overnight. Various portions of the chromatogram were eluted 4 times with 5 ml. portions of either distilled water or 0.25% acetic acid. The centrifuged material was made isotonic with sodium chloride before injection.</p> <p>Other drugs tested were ACTH, commercial epinephrine, histamine acid phosphate, substance P, and Pitressin. Pitocin and Protopituitrin. These were injected I. V. Epinephrin and ACTH were also given intraperitoneally. Pitressin in oil was injected subcutaneously.</p>	<p>Injected intravenously.</p>	<p>Rats with lesions.</p> <p>hypothalamic</p>	<p>ACTH releasing (adrenal ascorbic acid depletion) activity was found in eluates from that portion of the chromatogram with Rf 0.3 to 0.8. The eluates had also pressor factor. Eluates from Rf 0.0 to 0.3 were ineffective. The fraction with Rf 0.8 to 1.0 was also ineffective.</p> <p>Pitressin leads to ACTH release in rats treated with nembutal-morphine or with hydrocortisone overdosage or in rats with hypothalamic lesions. Histamine, epinephrine, substance P, and Pitocin were all ineffective in giving rise to ACTH release in rats with hypothalamic lesions.</p>



Authors and Year	Source and method of preparation of the extract.	Route of administration.	Dose and type of receptor animal.	Response
1	2	3	4	5
Sayers, G. (1957)	<p>Median eminence and ventral hypothalamus of calf brains. The tissues were as far caudal as the mamillary body and was cut at a depth of about 5 mm. The tissue was frozen in dry ice and extracted with 0.1 N HCl, heated at 100°C for 10 minutes and centrifuged. The other types of extracts used are:</p> <p>(a) 0.2 N Acetic acid extract of ventral hypothalamus.</p> <p>(b) Neutral saline extract of ventral hypothalamus, at room temperature.</p> <p>(c) Neutral saline extract of ventral hypothalamus, 4°C.</p> <p>(d) Oxycellulose eluate acetic acid extract of ventral hypothalamus.</p> <p>(e) Oxycellulose eluate acetic acid extract of ventral hypothalamus—lyophilized.</p>	<p>Injected into a saphenous vein over 5 minutes.</p>	<p>Two ml. extract equivalent to one ventral hypothalamus. A neck clamp was applied to the rat at the end of injection and 4 ml. blood was taken from the abdominal aorta for ACTH analysis. Blood ACTH was determined after injection of hypothalamic extracts in decerebrate adrenalectomized and in adrenalectomized and nembutal-morphine blocked rat. (Plasma free corticosteroids). Hypophysectomized rats.</p>	<p>After injection of the hydrochloric acid extracts there was a significantly higher level of ACTH in the blood of adrenalectomized decerebrate rats than in the blood of adrenalectomized hypophysectomized rats. This means that these extracts had a substance which stimulated the pituitary to release ACTH. The acetic acid extract was also active. The neutral extracts had no activity. Extracts No. (d) and (e) were also active.</p>





Authors and Year	Source and method of preparation of the extract.	Route of administration.	Dose and type of receptor animal.	Response
1		3	4	5
Clayton, G. W., and others (1957)	Chromatographic fraction of oxyelulose-washed protopituitrin and identified as D. Electrophoretically purified fraction D $\Delta$ .	(a) I. V. perfusion in 150 to 200 ml. saline for 4 hours. (b) Rapid I. V. injection.	(a) 5 to 10 mg. to 14 children (age 4 to 16 years) convalescent from various conditions. Plasma free 17-hydroxycorticoids were estimated after Nelson and Samuels at 0, 2, 4 and 6 hours. Urinary 17-OHCS were also studied. Controls—on 8 patients with similar perfusions of saline or with inactivated fraction D (by alkaline hydrolysis). (b) 25 to 45 micrograms of electrophoretically purified fraction D $\Delta$ in 1 to 2 ml. saline—rapid I. V. injection to 5 adult healthy male subjects. Plasma free 17-OHCS concentration at 0, 15, 30 and 60 minutes. Controls—inactivated fraction D $\Delta$ .	(a) Rise in plasma (4 hours) and urinary 17-OHCS. (b) Increase in plasma 17-OHCS after 15 minutes with a gradual decline over 60 minutes. No objective change in B.P., pulse, pulse pressure, cornea or skin colour, skin moisture, respiration rate and temperature. No feeling of general discomfort, nausea, intestinal cramp, or headache. With D $\Delta$ there was mild sensation of warmth in the face for a few seconds. Conclusion: A non-pressor material extracted from protopituitrin, known to stimulate release of ACTH <i>in vitro</i> was shown to stimulate release of ACTH in human subjects as evidenced by measurements of plasma 17-OHCS. This increased secretion of ACTH was obtained in absence of significant side effects.
de Wied, D., Bouman, P. R., and Smelik, P. G. (1958).	A non-saponifiable lipide extract was prepared after the method of Slusher and Roberts (1954) from fresh ovine posterior hypothalamus. Of this extract 10 mg. was suspended in saline just before injection.	The extract was injected intra-peritoneally.	Extract—0.2 ml. per 100 gm. body weight. Pitressin was injected I. V. into the tail vein 0.2 ml./100 gm. body weight containing 0.2 I. U. Receptor animals were: (1) normal and hypophysectomized rats.	Lipide extract requires the intactness of hypothalamic structures for its action. Pitressin contains the specific neurotransmitter.



Authors and Year	Source and method of preparation of the extract.	Route of administration.	Dose and type of receptor animal.	Response
1	2	3	4	5
Schally, A. V., Saffran, M., and Zimmermann, B. (1958).	<p>Acetone dried powders of neurohypophysis, hypothalamic or brain tissue were extracted with hot aq. 0.5% acetic acid. The material was precipitated with methanol.</p> <p>Protopituitrin treated with oxycelulose resulted in a reduction to one-tenth in the ACTH content. Serial paper chromatography in four different solvent systems (Butanol-acetic acid-water, m-Cresol-saturated with water, Acetone-water, Propan-1-ol-water) has been done to the concentration of the CRF in Protopituitrin. Purified CRF has the chemical and chromatographic properties of a peptide, which are separate from oxytocin and vasopressin. After acid hydrolysis of purified CRF, the following amino acids were found: cysteine, aspartic acid, glutamic acid, glycine, proline, lysine, phenylalanine, alanine, serine and histidine.</p>		<p>(2) rats with :  (a) nembutal  (b) nembutal and morphine  (c) hydrocortisone.  (3) rats having hypothalamic lesions.</p>	<p>Neurohypophysis and hypothalamic extract contain a corticotrophin releasing factor (CRF) and it stimulates the release of ACTH from isolated anterior pituitary tissue.</p>



Authors and Year	Source and method of preparation of the extract.	Route of administration.	Dose and type of receptor animal.	Response
1	2	3	4	5
Curri, S. B. (1958)	Bovine anterior and posterior hypothalamus and cerebral cortex were extracted after the method of Slusher and Roberts (1954), suitably modified. The hypothalamic lipid extracts were composed of 71.88% of saturated and unsaturated phosphatides, 23.84% of sterols and cholesterol esters, 4.28% of neutral fats and other lipids (unidentified fraction of Brante) per 100g. of dry substance. The diencephalic region is particularly rich in unsaturated phosphatides compared to the cerebral cortex. The lipid fraction has not got any antidiuretic power.	Intramuscular and intravenous route.	2.5 mg. to 5 mg. per 100 g. body weight in mice, rats, hamsters, cats, rabbits and dogs for 7 to 15 days according to the animals' size. Higher doses (from 10 to 25 mg. of dry substance) were also used.	With small dose there was a release of neuro-secretion from nuclei supraopticus and para-ventricularis. This coincided with cellular hyperfunction. There were basophilia in the adeno-hypophysis, hyperplasia of the follicular epithelium of the thyroid, hyperplasia of the zona fasciculata of the adrenal, and modifications in the pancreas and gonads. With higher dose of the extract there was accumulation of neurosecretory material in the nucleus supraopticus and para-ventricularis and in the infundibular region. There was hypofunction in all endocrine glands.
Privat, de Garilhe, M., Gros, C., Chauvet, J., Gromagot, Cl., Mialhe-Voloz, C. and Benoit, J. (1958).	Neurohypophyseal extracts were fractionated on IRC-50 ion exchange resin. The active fractions are different from vasopressin.			The fractions when added to incubated pituitary glands lead to an increase in ACTH and there was adrenal ascorbic acid depletion when injected into steroid blocked rats.



Authors and Year	Source and method of preparation of the extract.	Route of administration.	Dose and type of receptor animal.	Response
1	2	3	4	5
de Wied, D. (1958)	Non-saponifiable lipids extract of bovine posterior hypothalamus was prepared after the method of Slusher and Roberts (1954).		(a) normal rats (b) hypophysectomized rats. (c) nembutalized rats (d) rats with nembutal and chlorpromazine treatment. (e) nembutalized rats treated with dihydroergotamine, atropine sulfate, neo-atropan or hexamethonium chloride.	The effect of the extract depends on the function of hypothalamic centers. Their function might be abolished by chlorpromazine treatment.
Royce, P. C. and Sayers, G. (1958)	(a) Acid extracts of calf hypothalamus (stalk median eminence area)=crude extracts. (b) Oxycellulose fractions. (c) Pepsin treated extracts.	Intravenous infusion over 5 mins.	Acute median eminence lesioned rats. Dose varied from .025 to .4 SME unit. (Each piece of tissue has been designated a stalk median eminence unit (SME unit).)	Depletion of adrenal ascorbic acid is due in large measure to a pepsin labile factor different from ACTH and from vasopressin.
Safran, M., and Schally, A. V. (1958).	By an <i>in vitro</i> system, the control of the release of ACTH from the anterior pituitary has been studied. Isolated rat anterior pituitary tissue is used. ACTH released in the incubation medium of the anterior pituitary tissue is assayed by its corticosteroidogenic effect on rat adrenals <i>in vitro</i> .			Minor increase in the release of ACTH has been observed by epinephrine and norepinephrine. "Hypothalamus, brain cortex, and posterior pituitary tissue have little, if any, influence by themselves, but in combination with norepinephrine, these tissues stimulate the release of ACTH. The greatest effect is exerted by the posterior lobe. The active material in the posterior lobe seems to be similar to, but distinct from, the beef posterior lobe principle, oxytocin and vasopressin".



Authors and Year	Source and method of preparation of the extract.	Route of administration.	Dose and type of receptor animal.	Response
1	2	3	4	5
Loeman, S. E. and Voelkel E. F. (1959).	Calf hypothalamus including the median eminence was homogenized with 5 ml. of 0.1 N HCl per gm. of tissue at 4°C for 30 seconds. The lyophilized supernate was ground with 20 ml. of ice-cold distilled water per gm. and the soluble fraction was tested.	Intravenous injection.	0.15 to 0.30 mg. N. Intact rats anaesthetized with pentobarbital and treated with morphine and rats pre-treated with cortisol, 10 mg./kg. and hypophysectomized rats were receptor animals.	A fall of more than 100 mg. % in the adrenal ascorbic acid. The extracts were also effective in coliced blocked rats. About one fourth of the total effect could be due to ACTH or like material as tested in hypophysectomized rats. The amount of vasopressin in the extracts was too low—0.05 unit/dose.
Rochefort, G. J., Rosenberger, J., and Saffran, M. (1959).	The CRF preparations were purified from posterior pituitary extracts. The 'standard' preparation was obtained at the second m-cresol state in the paper chromatographic purifications procedure and was concentrated about 170 times over the starting extract. The 'purified' preparation was obtained at the final propanol stage and was concentrated about 5000 fold. Before injection these materials were diluted with 0.5% acetic acid.	Intravenous and intra-peritoneal.	Rats. 1 microgram of 'standard' CRF was injected via a superficial leg vein.	There was a sharp fall in the corticotrophic content of the anterior lobe of pituitary at 30 min. It was 50% of the control in 120 min. In the posterior lobe there was a sudden fall to 50% of control at 60 min. This came to normal at 120 min. Changes in the adrenal ascorbic acid in these animals were similar to those in the corticotrophin content of the anterior lobe. There was a fall of 50% in the corticotrophin activity of the anterior lobe.
			Intraperitoneal injection of 4 to 20 micrograms of the standard CRF or of 1.5 micrograms of the purified material.	



Authors and Year	Source and method of preparation of the extract.	Route of administration.	Dose and type of receptor animal.	Response
Guillemin, R., E., Dear, W., Nichols, B. Jr. and Lipscomb, H. S. (1959)	(a) CRF preparation used was fraction D prepared and characterized <i>in vitro</i> according to Guillemin <i>et al.</i> (1957). This fraction had a vasopressor activity of 0.4 U/mg.  (b) Lysine vasopressin. Purified vasopressin was further purified by partition chromatography on paper with system m-cresol/H <sub>2</sub> O. After elution, the lysine vasopressin (Rf: 0.82) had a specific activity of 287 U/mg.	Intravenous injection.	ACTH release (plasma free corticosteroids and adrenal ascorbic acid depletion) in morphine-naloxone blocked rats and rats with effective hypothalamic lesions.  Dose: Amount of CRF fraction D injected in 20.0 micrograms to 320.0 micrograms with pressor equivalent 8 mU to 128 mU. Pressor amount of Lysine vasopressin in mU 4 to 128.	CRF preparation fraction D stimulates release of ACTH in animals with hypothalamic lesions or pharmacological blockade. The doses of fraction D were where pressor equivalents as pure lysine vasopressin were inactive. The hypophysiotropic activity of fraction D is due to a substance different from vasopressin.
McCann, S. M. and Haberland, P. (1959).	(a) Beef cortex and stalk-median eminence tissue were placed in beaker on dry ice. Tissues from 20 brains weighed, 40 ml. of 0.2 M acetic acid added and the mixture homogenized for 120 minutes. The mixture was refluxed for 10 minutes in 100° C water bath and then centrifuged for 1 hour. The supernatant injected.	I.V.	Acute lesions of the median eminence of the tuber cinereum. Rats drinking at least 100 ml. of water during the first 24 hours post-operatively were selected for assay procedure. Assay was done 48 hours after lesions.  Dose: ACTH releasing activity of extracts of beef brain.	Extracts of SME of beef or rat brain give rise to specific release of ACTH (adrenal ascorbic acid depletion) in rats with hypothalamic lesions. The major portion of this effect is due to a CRF. Ten to 20 per cent. of these extracts appear to be due to vasopressin.





Authors and Year	Source and method of preparation of the extract.	Route of administration.	Dose and type of receptor animal.	Response
1	2	3	4	5
	(b) Rat stalk-median eminence tissue or brainstem was taken from hypophysectomized rats 24-48 hours after hypophysectomy and ground in 0.5 ml. of 0.1 N HCl. One-tenth N HCl was added to pools of 6-12 stalk-median eminences to bring the volume to 0.5 ml. per stalk-median eminence. Mixture centrifuged for 15 minutes and the supernatant injected into assay rats.		<i>Stalk-median eminence:</i> .0125, .0032 and .0016 units (1 U is one stalk median eminence or equivalent weight of cortex). ACTH releasing activity of extracts of rat brain. SME—1 U 2 U Brain stem—2 U 1 U is one SME.	
Roy, B. B. (1959)	Hypothalamic extracts and extracts from cerebral cortex have been taken from normal, stressed, hypophysectomized stressed dogs in different post-stress days and the effect of these extracts on the adrenal venous blood 17-OHCS output has been noted. The extracts have been prepared with acid alcohol or trichloroacetic acid.	Intra-carotid injection.	The assay animals (dogs) have been prepared by exteriorization of the carotid artery and the animals have been conditioned for blood removal or intra-carotid injection of blood. Cannulation of the right adrenal vein has been performed before the experiments for collection of adrenal venous blood. The extracts have been injected into the carotid artery of the assay dogs and ventral hypothalamectomized assay dogs. Blood 17-hydroxycorticosteroid has been estimated after the method of Silber and Porter (1954).	Hypothalamic extracts from normal dogs could elevate 17-OHCS output in the adrenal vein. With the injection of hypothalamic extracts from dogs with burn, fracture and intestinal obstruction, the percentage rise in 17-OHCS output in the adrenal venous blood was good and it was better than that found after injection of the hypothalamic extracts from normal dogs. The maximum response was noted after the injection of the hypothalamic extracts from burned dogs (1st day of burn). The percentage rise in 17-OHCS output in the test dogs varied when the hypothalamic extracts from dogs in different post-stress days were tested. Good response was achieved

#### Dose:

Extracts of hypothalamus from two dogs comprised each group. Blood was



Authors and Year	Source and method of preparation of the extract.	Route of administration.	Dose and type of receptor animal.	Response
1	2	3	4	5
			collected from adrenal vein 20 minutes after injection of the extract.	when the hypothalamic extracts were taken from hypophysectomized dogs (1st and 3rd day of hypophysectomy) and injected into the carotid artery of the test dogs. Burn trauma applied to the hypophysectomized dogs (1st day of hypophysectomy) could increase the potency of hypothalamic extracts. When the hypothalamic extracts were taken from hypophysectomized dogs on the ninth day of hypophysectomy and injected into the assay dogs, the response was lower than that found after injection of the hypothalamic extracts from the normal dogs. With cortisone treatment in the hypophysectomized dogs there is lowered potency of the hypothalamic extracts when they are tested in the assay dogs. Extracts from the cerebral cortex could increase 17-OHCS output, but the magnitude of response was much lesser than that found after injection of the hypothalamic extracts. Presence of the hypophysis in the assay animals is essential for the response. Anaesthesia with pentobarbital sodium and hypophysectomy could not block the response.





Authors and Year	Source and method of preparation of the extract.	Route of administration.	Dose and type of receptor animal.	Response
1	2	3	4	5
Roy, B. B. (1959)	Blood was collected from the pituitary fossa after extirpation of the pituitary of normal dogs and dogs with burn, fracture and intestinal obstruction (blood was similarly collected in different post-stress days).	Intracarotid injection.	25 ml. blood was injected into the exteriorized carotid artery of non-stressed dogs and the percentage rise in 17-OHCS output in the adrenal venous blood was noted.	There was 185.7% rise in 17-OHCS output in the adrenal venous blood of the assay dog when blood was collected from the pituitary fossa after extirpation of the pituitary of a normal dog and injected into the carotid artery of the assay dog. Blood similarly collected from the stressed dogs and injected into the assay dogs could increase 17-OHCS output in the adrenal venous blood of the latter. There was 325% rise when blood was collected from the pituitary fossa on the first day of burn and tested. It was 302% on the fifth day of burn. With fracture, the value was 205% on the first day and 82% on the fifth day. It was 225% on the first day of intestinal obstruction and there was a further rise to 300.2% on the fifth day.

Increase plasma corticosteroids.

Morphine-pentobarbital blocked rats.

Schally, A. V. and Guillemain, R. (1959). Chromatography of neurohypophyseal extracts on carboxymethyl-cellulose gives rise to a protein fraction. The authors think that the CRF activity is due to a polypeptide which is distinct from vasopressin.



Authors and Year	Source and method of preparation of the extract.	Route of administration.	Dose and type of receptor animal.	Response
1	2	3	4	5
Royce, P. C. and Sayers, G (1960).	Calf median eminence and pituitary stalk (SME) fractionation of the crude acetic acid extract on carboxymethylcellulose columns gives rise to the preparation (partially purified CRF).	Intra-venous route. Infusion time = over 30 seconds.	Assays for CRF activity were done in median eminence lesioned rats (250 to 300 g). Corticotrophic activity of the extracts was tested by assay in 24 hours—hypophysectomized rats (110 to 135 g). Vasopressin content was estimated by pressor response of rats pretreated with phenoxybenzamine. The dose of each fraction used in the three assay systems was equivalent to 0.3 SME.	The partially purified extract leads to adrenal ascorbic acid depletion in median eminence lesioned rat. This CRF is substantially free from the corticotrophic and pressor activities.
de Wied D. (1961).	The extracts were prepared from whole hypothalamus and frontal lobes of rats hypophysectomized 24 hours previously. Pooled tissues of 20 animals were ground with 1 ml. of 0.1 N HCl per 200 mg. of fresh material in a mortar with sand. This was centrifuged for 10 minutes. The supernatant was brought to pH 7 with 0.1 N NaOH before injection.	Intra-venous injection.	20 mg. hypothalamus or cerebral cortex extract per 100 grams of body weight of rats with lesions in the median eminence, 18 hours after electrocoagulation.	<i>In vitro</i> steroidogenesis by adrenal glands was used as an index of pituitary-adrenal activity. The blocking effect of the lesion on ACTH release due to stress was measured by the effect of ether anaesthesia on corticoid production <i>in vitro</i> of left adrenal, whereas steroidogenesis of the right adrenal was used as an index of corticotrophic effect of CRF. CRF is present in the extracts.





Authors and Year	Source and method of preparation of the extract.	Route of administration.	Dose and type of receptor animal.	Response
1	2	3	4	5
Eik-Nes, K. B., Brown, D. M., Brizzee, K. R. and Smith, E. L. (1961).	Spinal fluid from third ventricle and the praesoptic subarachnoid region of normal, & anaesthetized dogs. Human spinal fluid by spinal tapping. The factor in the spinal fluid can be purified approximately 25-fold by zone electrophoresis on a starch column or on paper. This factor seems to be protein or polypeptide in nature.	Intra-carotid injection.	The receptor animal is a trained dog with externalized carotid artery. 5 ml. spinal fluid either from dog or man was injected. 2 microgram partially purified spinal fluid protein per pound in 5 ml. of 0.85% NaCl.	Intracarotid injection of C. S. fluid either from man or from dog leads to a rise in plasma 17-OHCS within 15 minutes. The purified factor showed similar response. When the materials were injected through leg veins no significant alteration in the level of plasma 17-OHCS was noted.
Brizzee, K. R., and Eik-Nes, K. B. (1961).	Dog's brain was used as source of the extract. The animals were anaesthetized with sodium pentobarbital (35 mg./kg. I.V.): Cylindrical "punch outs" from both frozen (liquid nitrogen) and unfrozen hypothalami were taken. Tissues were obtained from nucleus supraoptics, nucleus lateralis, nucleus paraventricularis, anterior median eminence, optic tract, mammillary body and cerebral cortex (posterior sigmoid gyrus). The average wet weight of each punchout was about 2 mg. and total weight of about 50 mg. of tissue comprised a sample. Saline extract was used (1 ml. saline per mg. of tissue). Protein content was 50-100 micrograms per mg. of brain tissue.	Extract injected into exteriorized carotid artery of trained non-stressed dogs.	Supernatant fluid from 1 mg. tissue was diluted to 5 ml. with 0.85% NaCl and injected into dogs.	Mean peripheral blood levels of 17-hydroxycorticosteroids increased significantly within 15 minutes after injection of the extracts of nucleus supra-opticus and anterior median eminence. Extracts from other structures when injected did not show any significant rise in plasma 17-hydroxycorticosteroid level when compared to the controls. There was also no significant difference in the responses obtained after injection of extracts made from frozen or unfrozen tissues.



Authors and Year	Source and method of preparation of the extract.	Route of administration.	Dose and type of receptor animal.	Response
1	2	3	4	5
Rumsfeld, H. W. (Jr.) and Porter, J. C. (1962).	Extracts and purified fractions from 600 bovine posterior pituitaries and arginine vasopressin.		<p>Pressor activity was assayed in dibenzylidene treated rats. Adrenal ascorbic acid depleting activity was assayed in hypophysectomized rats and cortisol-depressed rats. ACTH-releasing activity = Adrenal ascorbic acid depleting activity in cortisol depressed rats minus AAA depleting activity in hypophysectomized rats.</p> <p>The ratios of pressor activity to ACTH releasing activity in the acetic acid extract, trichloroacetic acid extract, fraction 12 from ion-exchange column, and arginine vasopressin were 170/1, 166/1, 235/1, and 164/1 respectively.</p>	
Leeman, S. E., Glenister, D. W., and Yates, F. E. (1962).	Calf hypothalamus including the median eminence was homogenized in a blender for approximately 25 seconds at 4°C with 5 ml. 0.1N HCl per gram of wet tissue. The homogenate was centrifuged at 4°C for 1½ hour. The supernatant was lyophilized and the powder kept at 4°C in a desiccator.	Intravenous 0.2 ml. 0.4 ml.	Morphine or corticosterone treated rats.	ACTH secretion was noted by increase in plasma corticosterone level or by adrenal ascorbic acid depletion. Morphine or corticosterone could not block ACTH release after injection of hypothalamic extract. The extract was free from significant ACTH contamination. The hypothalamic extract possesses a unique ACTH-releasing activity.
Brodish, A. and Long, C. N. H. (1962).	Peripheral blood of hypophysectomized rats.			ACTH releasing substance observed in the peripheral circulation of hypophysectomized animals is a specific hypothalamic factor for the regulation of anterior pituitary ACTH secretion. Hypothalamic lesioned rats had no such substance in the peripheral blood.



Authors and Year	Source and method of preparation of the extract.	Route of administration.	Dose and type of receptor animal.	Response
1	2	3	4	5
Guillemin, R., Schally, A. V., Lipscomb, H. S., Anderson, R. N., and Long, J. M. (1962)	Extracts of hog hypothalamus were chromatographed on carboxymethylcellulose.			The following substances were detected: Oxytocin, $\beta$ -MSH, $\alpha$ -MSH, lysine vasopressin, $\beta$ -CRF, $\alpha$ -MSH like or ACTH-like peptide, and ACTH.
Schally, A. V., Lipscomb, H. S., Long, J. M., Dear, W. E., and Guillemin, R. (1962)	Extracts of the hypothalamus of dogs (nembutal anesthesia) were chromatographed on carboxymethylcellulose.			The following substances were identified: Oxytocin, $\beta$ -MSH, arginine vasopressin, CRF, $\alpha$ -MSH-like or ACTH-like peptide and ACTH.
Doepfner, W., Stürmer, E., and Berdo, B. (1963)	Mammalian neurohypophyseal hormones and some related synthetic analogues and homologues.	Injected into external jugular veins of rats.	Pentobarbital blocked rats. The amount of a peptide which gave rise to 100% increase of plasma corticosterone was equivalent to one CR unit.	Pressor and CR activities of peptides related to the neurohypophyseal hormones need not be strictly correlated.
Critchlow, V., Lipscomb, H. S., and Guillemin, R. (1963)	Fraction D prepared after the method of Guillemin and others (1957).	(a) Subcutaneous injection in chronic experimental procedures. (b) Intravenous injection in acute experiments.	(a) Hypophysectomized female rats with autotransplanted anterior pituitary under the left renal capsule. 250 microgram/rat for 12 days, every six hours. (b) Intravenous injection of 100 microgram of CRF fraction D in 1.0 ml. physiological saline.	Prolonged administration of CRF has maintained synthesis of ACTH by the transplanted pituitary and its ability to release ACTH when acutely stimulated by CRF.
Recep, C. (1964)	Hypothalamic extract from pig, virtually free from corticotrophin and vasopressin. 250 microgram per ml. in 0.01N acetic acid.	Intravenous into the tailvein of mice.	Dexamethasone-blocked mice. Dose of CRF = 50 microgram.	Increased activation of the pituitary-adrenal system.





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PART III

COMPARATIVE STUDIES ON STRESS  
AND BRAIN MECHANISMS IN  
DIFFERENT SPECIES



## CHAPTER—1

### STRESS AND NEUROSECRETION IN THE EARTHWORM

#### *PHERETIMA POSTHUMA*

(Experimental Surgical Studies)

(1969)

In the posterosuperior portion of the supraoesophageal ganglion of the oligochaetes there are many neurosecretory cells (Scharrer, 1937 ; Harms, 1948 ; Hubl, 1953, 1956a, b ; Herlant-Meewis, 1955, 1956a, b ; Brandenburg, 1956 ; Otremba, 1961).

In the supraoesophageal and the suboesophageal ganglia and the first two ventral ganglia of the *Lumbricus*, a-cells and the blue cells have been demonstrated by the azan technique. Cells stainable with paraldehyde fuchsin have also been noted.

In *E. foetida* a-cells, b-cells, and "large and medium neurones" have been described by Herlant-Meewis (1955, 1956a, b). The location of the a-cells is in the posterosuperior portion of the cerebral ganglia. The a-cells have very dense cytoplasm and moniliform axons. The small and fusiform type b-cells are found in a group near the exit of the anterodorsal connective. The neurones also show neurosecretory phenomena. The product in the a-cells and their axons is stainable with paraldehyde fuchsin and chrome haematoxylin. Some cells only take up the counterstain. This difference in staining reaction is thought by Herlant-Meewis to indicate a secretory cycle. The acidophilic, PAS-positive product having no affinity for paraldehyde fuchsin is the starting point and ends in the stage where the product is cyanophilic, PAS-negative and paraldehyde fuchsin positive. In the final stage the product is stainable in the axons and they look moniliform. However, no secretory product has been noted by her in the axons of the b-cells.

Hubl (1953) identified ordinary ganglionic cells, azocarminophilic a-cells and the cyanophilic b-cells. Hubl (1956b) identified vacuolated cells which he called c-cells in connection with wound healing and regenerative process.

Bern and Hagadorn (1965) stated the possible equivalence of neurosecretory cell types described by different investigators including physiologic correlates in Oligochaeta.

Six cell types were described by Brandenburg (1956) in *Lumbricus terrestris* but he thought that they represented the secretion cycle of a single group of cells. Scharrer and Brown (1962) came to the conclusion that the differentially coloured neurosecretory cells situated posterosuperiorly in the supraoesophageal ganglion of the earthworm indicated different functional states of one particular cell type. They did not consider the existence of several different kinds of cells. In this connection the classification of Herlant-Meewis is advantageous to accept as she does not



attach too much importance to tinctorial properties but she relies more on anatomical features correlating with physiological observations.

The classification of cell types described by Herlant-Meewis has been adopted in this present investigation with a view to study the neurosecretory cells in the supra- and the subpharyngeal ganglia of *Pheretima posthuma* with special reference to (a) growth, (b) reproduction, (c) regeneration of body segments after amputation, (d) regeneration of the brain after extirpation, and (e) salt stress.

### MATERIALS AND METHODS

Immature, sexually mature and adult *P. posthuma* were collected for general histological studies throughout a year. For gonadectomy and brain removal, absolute alcohol and water mixture (6 : 100) has been used as an anaesthetic agent (Hubl, 1951). Fixation of the worms has been done in Bouin and sections have been stained by Gomori's chrome alum haematoxylin phloxine method and paraldehyde fuchsin. Masson's trichrome stain has also been used.

### RESULTS AND DISCUSSION

The nervous system of *Pheretima posthuma* consists of a pair of supra-pharyngeal ganglia (the brain or cerebral ganglia) (Fig. 1), a pair of subpharyngeal ganglia, peripharyngeal connectives (Fig. 2) and the ventral nerve cord. A nerve ring is formed by the suprapharyngeal ganglia, the subpharyngeal ganglia and the peripharyngeal connectives. The cerebral ganglia are situated in the third body segment and the subpharyngeal ganglia in the fourth segment. The ventral nerve cord is double but appears to be single. In the middle of each segment of the body the cord has a ganglionated swelling. From each cerebral ganglion there are cerebral nerves. They have sensory and motor fibres. There are afferents from the epidermis of the prostomium and from the wall of the buccal cavity. The brain is well vascularised and the interior of the ganglia gets good blood supply.

The nerve cells are peripherally situated in the brain having a central neuropile. Neuroglia cells and fibres are also noted. In the postero-superior portion of the brain there are type-a neurosecretory cells (Fig. 3) and their beaded axons demonstrable by Gomori's chrome alum haematoxylin stain and by aldehyde fuchsin stain. Some of these cells are not stainable by these methods, rather they take up the counterstains. Small fusiform b-cells (Fig. 4) have been noted near the junction of the brain with the connectives. The axons of these cells do not contain any neurosecretory substance. In the subpharyngeal and the segmental ganglia there are neurosecretory substance from the a-cells and their axons. The



changes in the b-cells are minimum. In the winter the a-cells and their axons are loaded with neurosecretion (Fig. 5).

TABLE I

Showing neurosecretory phenomena in *P. posthuma*

<i>P. posthuma</i>	Type-a cells	Type-b cells	Neuropile
Sexually Immature	+	few	Neurosecretion along axons of a-cells is scanty.
Sexually mature	++	+	Neurosecretion along axons of a-cells is well visualized.

The number and activities of the type-a cells increase with the advent of sexual maturity. Similar observation has been made by Herlant-Meewis (1956b) in relation to the postembryonic development of *Eisenia foetida*. Counting and measurement of cells have been done by Ogawa (1939) in *Pheretima communissima*. The ganglion cells in ventral and in the suboesophageal ganglia in the adult is nearly double in comparison to those in a newly hatched worm. About 3.5 times increase in the cell population was noted in the segmental ganglia in the eighteenth segment containing the prostate gland. About fifty-times increase was noted for the characteristic cells in the brain.

Heightened activity of a-cells during summer and rainy season and with the progress of sexual maturity leads one to think that these cells are probably engaged in someway with reproduction in *P. posthuma*.

#### NEUROSECRETION AND REPRODUCTION

In *P. posthuma* there are two pairs of testis and a pair of ovary. The testes are enclosed in a pouch in segments ten and eleven. On the posterior surface of the septum separating the segment 12 from the segment 13, the ovaries are situated. The gonadectomy has been performed ventrolaterally under anaesthesia.

In the first week after gonadectomy the b-cells are active, showing loss of neurosecretion and vacuolation. Following this there is accumulation of colloid in a-cells studied upto 4 weeks (Figs. 6, 7, 8, 9). At nearly two months when the worm becomes normal, depletion of neurosecretory substance in a-cells has been noted. Hubl (1953) noted changes in the a-cells after gonadectomy in *L. terrestris*.

Herlant-Meewis (1956a, b) noted arrest of oviposition, loss of weight and regression of secondary sex characters after cerebral ganglionectomy. Reproductive function begins after about eight weeks. In *Pheretima posthuma* similar observations have been made and the time for cerebral regeneration is about one month after brain removal and full reproductive function begins in the third month.



## BRAIN REGENERATION

After removal of the brain in *P. posthuma*, full reorganization of the lost brain takes place within a month. The newly formed brain in most worms is in the usual location; but sometimes it shifts to either right or left of the midline. In a few specimens the brains are of unequal size.

STAGES IN THE REGENERATION OF THE BRAIN IN *P. posthuma*

The axons of the sensory cells in the prostomium sprout towards the area which was occupied by the cerebral ganglia and they form two masses. Neurones, neurosecretory cells and neuroglia develop from undifferentiated cells surrounding the masses (Fig. 10).

Hubl (1956) and Herlant-Meewis (1962) described these changes in detail. Schwartz (1932) observed replacement of the brain of *lumbricus* within about three weeks. These worms with regenerated brains did not burrow, but they moved about with the anterior ends raised. The new brain developed from differentiation of connective tissue cells.

## REGENERATION OF LOST SEGMENTS AND THE INFLUENCE OF NEUROSECRETION

The presence of nervous system is essential for the segmental regeneration (Carter, 1940). After amputation of the anterior sixteen segments in *L. terrestris*, regeneration did not take place (Harms, 1948); but regeneration of a few segments was observed after implantation of a cerebral ganglion. From the experiments of Hubl (1956) it is evident that the presence of cerebral ganglia is necessary when the posterior regeneration is starting. The process of posterior regeneration continues when the cerebral ganglion is removed 48 hours after the amputation of the posterior part of the body. Removal of suboesophageal ganglion and the first five ganglia of the ventral chain inhibits posterior regeneration.

PERSONAL OBSERVATION IN *P. POSTHUMA*

(a) 2-3 days after amputation of ten posterior segments there was increased activity of the a-cells in the form of loss of neurosecretory material and formation of vacuoles. The same feature was noted in the subpharyngeal ganglia.

(b) Three weeks after the posterior amputation the last segmental ganglion still showed increased activity (Figs. 11, 12).

(c) In the first week after posterior amputation the b-cells showed vacuolation also.

(d) The brain of an earthworm is taken three days after amputation of last ten segments and implanted into the body-cavity of another brainless *P. posthuma* with loss of last ten segments. Regeneration happens. During three days the donor earthworm produces sufficient quantity of hormone which can help in posterior regeneration in the recipient earthworm in the absence of the brain.



### SALT STRESS AND NEUROSECRETION

When *P. posthuma* is kept in 0.9% sodium chloride solution for six hours, the a and b cells of the suprapharyngeal ganglion show loss of neurosecretory material. There is loss of neurosecretory material from the subpharyngeal neurosecretory cells. The neurosecretory cells of the segmental ganglia show loss of cell outline and confluence of neurosecretory materials and vacuolation (Figs. 13, 14).

### CONCLUSIONS

(1) Type-a neurosecretory cells are found in the posterosuperior portion of the brain in *Pheretima posthuma*. Small fusiform b-cells have been noted near the junction of the brain with the connectives. No neurosecretion is noted in the axons of b-cells.

(2) With growth and sexual maturity type-a cells increase more than type-b cells.

(3) During reproductive period depletion of neurosecretory substance and vacuolation is noted in type-a neurosecretory cells. In winter a-cells are loaded with neurosecretion.

(4) Cerebral ganglionectomy has its effect on reproductive physiology. Cerebral regeneration takes place within a month after brain removal and full reproductive function occurs in the third month.

(5) Mechanism of brain regeneration has been described in *Pheretima posthuma*. The undifferentiated cells differentiate into neurones, neurosecretory cells and neuroglia.

(6) Immediate response is noted in the a-cells after posterior amputation. Then the response is noted in b-cells. The last segmental ganglion also shows neurosecretory activity after amputation.

(7) The brain of *Pheretima posthuma* contains hormone for posterior regeneration. The brain is activated by trauma and peak activity is noted in the third day after amputation.

(8) Loss of neurosecretory substance has been noted in neurosecretory centers when the earthworm is kept in 0.9% sodium chloride solution for six hours.

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## CHAPTER—2

### NEUROSECRETION IN ACHATINA FULICA

1971

#### INTRODUCTION

Simpson *et al.* (1966a) surveyed the evidence for neurosecretion in *Gastropod Molluscs*. They classified the evidences into the following categories :—(1) neurosecretion to be morphologically described, (2) correlation of physiological changes with changes in secretory activity, (3) demonstration of a hormone produced by the specialized neurosecretory neuron, and (4) neurosecretory cells controlling synthesis and/or release of a hormone. Bern (1962) described three types of neurons : (1) *possible neurosecretory cells*, which contain cytoplasmic droplets, granules or vacuoles, (2) *probable neurosecretory cells*, which in addition to the characters described in (1), show the secretory cycle with discharge of secretory products or cellular changes at different periods of the year, and (3) *definite neurosecretory cells*, which secrete hormone. Boer (1965) utilized this classification for studying neurosecretion in *Lymnaea stagnalis* and Cook (1966) studied the morphology and histology of the central nervous system of *Succinea putris* (L).

In Basommatophora neurosecretory cells have been described by Gabe (1954), Lever (1957) and others. Lever (1957) noted five types of secretory cells :—cells A (subgroups A1, A2, A3), cells B, C, D, E. Joosse (1964) studied the neurosecretory phenomena in the cerebral ganglia of the basommatophoran Snail *Lymnaea stagnalis*, Mediodorsal cells and laterodorsal cells produce Gomori-positive neurosecretory material. The nsm is transported to the bulb-shaped endings at the periphery of the median lip nerves. The phloxinophilic nsm of caudodorsal cells is transported to the periphery of the cerebral commissure.

Neurosecretory cells have also been described in the *Stylommatophora*. Herlant-Meewis and Van Mol (1959) described neurosecretory cells in the posteromedial portion of the buccal ganglion of *Arion rufus* and *Arion subfuscus*. The nsm migrates along the axons. The neurosecretory fibres enter the posterior gastric nerve and spread into the connective tissue of the buccal ganglion where there is a neurohaemal area.

Van Mol (1960, 1967) further reported on the mesocerebral neurosecretory cells. The axons showing migrating neurosecretion form a bundle which ultimately divides into two. One crosses the midline and join with the bundle of the opposite side. The other bundle courses through the metacerebrum of the same side to form the nervus arteriae cerebialis which ends in the wall of the cerebral artery. There is cyclic activity in these cells. The mesocerebral neurosecretory cells show maximum activity during sexual maturity.



Krause (1960) described types I and II cells in the cerebral and visceral ganglion of *Helix*. The type II (Sackzellen) cells of the visceral ganglion controls hibernation by production of a hormone.

Simpson *et al.* (1966b) explored the relationship between the cerebral fuchsinophilic cells and epithelioid mediodorsal bodies of the basommatophoran pulmonate gastropod, *Helisoma tenue*. "Neurosecretory neuropil" was seen to be formed by extensive branching of neurites across the ganglionic capsule under the mediodorsal bodies. The structure of the follicle gland was suggestive to have non-endocrine function. The visceral fuchsinophilic cells send processes to end on the anterior aorta and within the perineurium of the nervus intestinalis.

Tentacular (optic) neurosecretory phenomena have been described by Tuzet *et al.* (1957), Lane (1962, 1963, 1964) and others.

The works of Hekstra and Lever (1960), Lever and Joesse (1961) and Lever *et al.* (1961) suggest that neurosecretory cells are engaged in the regulation of water equilibrium.

### MATERIALS AND METHODS

Adult specimens of *Achatina fulica* were collected locally and they were reared up in the usual laboratory conditions.

The brain and other ganglia were dissected quickly, fixed, and embedded in paraffin wax. Serial sections were cut at 5-7 $\mu$  thickness. Transverse and horizontal sections were made. Different fixatives were used *e.g.* Susa, Stieve, Bouin. The staining methods were: Gomori's CAHP stain, Gabe's paraldehyde fuchsin with or without counterstaining, Crossman's stain, PAS, Sudan black B, Luxol Fast blue (Kluver and Barrera), performic acid—alcian blue (Adams and Sloper).

For partial surgical excision of dorsal bodies and mesocerebral cells cautery was used and the techniques were as described by Joesse and Lever (1959).

### RESULTS

#### *Dorsal bodies :*

(A) This body is situated dorsal to the brain with extensions anteriorly, medially, laterally, and backwards. The part which is situated anteriorly and medially is maximum in front of the cerebral commissure. The lateral and posterior extensions are not demonstrable in more ventral and horizontal sections of the brain. Thus the shape of the body is not concentrated into a globular or concentrated mass as is demonstrated in Basommatophora (Fig. 1A & B). This body in *Achatina* is not divisible into medio or latero-dorsal parts. It does not communicate with the brain at any point of the attachment with the perineurium. In sections through more dorsal aspect of the brain (where the two halves of the brain are separate), the cells of the dorsal body are seen to be present in the chink and extend posteriorly and laterally. All the three elements—anterior, middle and posterior are seen to be continuous.

The cells of the dorsal body have spherical or oval nuclei with nucleoli.



Connective tissue fibres enter into the bodies from the perineurium of the cerebral ganglion.

(B) Extracerebral positions (around the visceral complex).

Cells akin to those of the dorsal bodies are found in the neurilemmal sheath of the visceral ganglion and in between other ganglionic masses of the complex (Fig. 2A, B). These cells have got the same tinctorial characteristics as those of the dorsal bodies. These cells do not take up the neurosecretory stains. Seasonal fluctuations have been noted in the cytoplasm/nucleus ratio; but one very peculiar feature is that these extracerebral bodies do not assume the particular pattern of cell-arrangement in different seasons of the year, as is noted in the cells of the dorsal bodies. These cells are of endocrine in nature and get stimulated/depressed by the neurosecretory material from the visceral complex, either by direct continuity or through vascular distribution, as nsm (fuchsinophilic) has been demonstrated between cell masses. The actual nature of these endocrine bodies is difficult to assess at present but that the seasonal fluctuation along with the condition of the ovotestis reminds one of their function as one of reproductive in nature.

(C) Duct systems as demonstrated by Cook (1966) in the dorsal bodies of *Succinea putris*.

In *Achatina* such duct systems which are open to the exterior and communicating with blood sinuses were not found. However, in the month of July and August the cells of the dorsal bodies (in horizontal section) show alveolar and tubular arrangement with open spaces in the centre. Connective tissue spaces are also there and some cells actually surround blood spaces having a chance for discharge of the cell contents (hormones) into the blood. In March and April the cells of the dorsal bodies assume a compact nature and the alveolar and tubular arrangement of the cells is lost.

*Buccal ganglion :*

The neurosecretory cells in the buccal ganglia can be stained with Sudan Black B or Luxol Fast blue. The stainable cells are situated dorsally and medially. The LFB positive substances can be noted in the periphery of the cell in the form of granules or flocculent masses (Fig. 3). Axonal migration was noted in some.

*Cerebral ganglion :*

The medium and small cells of the mesocerebrum take up chrome alum haematoxylin stain and they are also fuchsinophilic (Fig. 4A, B, C). The neurosecretory cells had high cystine content as there was strongly positive reaction when methods were used for demonstration of S-S bonds. Luxol Fast blue positive substances can be located in the cytoplasm. The "neurosecretory neuropil" as described by Simpson *et al.* (1966b) on the mediodorsal surface of the cerebral ganglion in *Helisoma tenue* has also been found in the same location in *A. fulica* i.e., at the junctional place between the dorsal body (Medial complex) and the mesocerebral cells (Fig. 5). The axonal migration of nsm could be demonstrated towards the cerebral commissure both anteriorly and posteriorly (Fig. 6). Migrations were also possible along commissural nerves. Short axons of some



neurosecretory mesocerebral cells had direct contact with the intracerebral blood vessels. Some mesocerebral cells send neurosecretory axons through the posterolateral part of the cerebral ganglion to the wall of the cerebral artery (Fig. 7).

#### *Visceral ganglion :*

In the visceral ganglion neurosecretory cells (medium and small sized) could be observed having tinctorial affinities for Gomori's CAH and aldehyde fuchsin. Sudan Black B positive and Luxol Fast blue positive cells were also noted (Fig. 8A, B). These included very big cells where LFB positive nuclear bodies could be seen. The medium and small sized cells had a single regular nucleus with a nucleolus. The big cells had large and irregular nuclei. The arrangement of the cells in the ganglion was peripheral with a central neuropil where fuchsinophilic droplets and LFB positive bodies could be found. Surrounding the visceral ganglion and in between other members of the complex there were small cells like those in dorsal body. Amongst these cells there were fuchsinophilic neurosecretory materials. This fuchsinophilic nsm could also be found along the axons of some medium sized cells. Their termination could not however, be traced for a long distance. On the medial face of the ganglion near the aorta there was a pool of fuchsinophilic nsm, which could serve as a neurohaemal area. The population of the neurosecretory cells in the visceral ganglion did not alter throughout the year.

#### *Experimental Surgical Studies of the Dorsal Body and Mesocerebral Cells :*

Selective injury to the mesocerebral cells was not possible without injuring the dorsal body and therefore the injury reactions were noted in both the places together.

In the mesocerebrum the damaged area was filled up by glia cells and there was connective tissue proliferation. The injured cells lost tinctorial affinity for neurosecretory material. Some axons intensely stained with Gomori's CAH and aldehyde fuchsin. The neighbouring uninjured mesocerebral cells showed evidences of injury stimulus. These cells had depletion of neurosecretory material and the axons were loaded with nsm. Such axons could be traced towards the neurohaemal areas.

Connective tissue proliferation could be noted in the injured dorsal bodies and connective tissue. Strands ran in different directions into the remaining cells of the dorsal bodies. Nearer to such strands the cell was bigger in size with a big and irregular nucleus. The tinctorial affinity of the cells did not change. There was abundance of nsm surrounding the hypertrophic cells.

### DISCUSSION

The dorsal body in *Achatina fulica* has been described and their cellular characters have been mentioned. Similar cells have been noted around visceral ganglion complex. These cells are endocrine in nature.

Seasonal variation in the cellular arrangement and maximum activity with the advent of the breeding season leads one to think about the production of a reproductive hormone from the dorsal body.



Joosse (1964) considered these bodies to be endocrine organs and probably they had a role in the process of ovulation and oviposition. Osmoregulatory function was ascribed to these bodies by Nolte (1965).

Joosse (1964) discussed previous works on mediodorsal bodies and their function. Bohmig (1931) noted cellular projections from dorsal cells in the dorsal bodies of *L. stagnalis* and *Planorbarius corneus* entering into the cerebral ganglion, after penetrating the connective tissue covering of the bodies. Dorsal bodies stored materials for transport to the ganglion cells along the projections of the "Dorsalzellen" and the glia cells. Such a contact between the C-cells of mediodorsal bodies and the neurosecretory cells in the cerebral ganglia of some pulmonates was also observed by Lever (1958); but the direction of flow was thought to be in reverse way i.e. from neurosecretory cells towards the C-cells of the mediodorsal bodies and so the C-cells could be influenced by the neurosecretory material. Joosse (1964) could not observe the elongated cytoplasmic processes of the C-cells of the mediodorsal bodies of *Lymnaea stagnalis*, to pass the fibrous membrane between the bodies and cerebral ganglion. The connective tissue fibres also did not cross the barrier in between the bodies and the cerebral ganglion. Cook (1966) did not find long cell processes from C-cells of the mediodorsal bodies in *S. putris*. The cells of the dorsal bodies in *Achatina fulica* also do not possess any cytoplasmic processes.

The buccal ganglia of *Achatina fulica* possess some neurosecretory cells in the dorsomedial part of the ganglion with migration of nsm along the axons towards posteroventral aspect of the ganglion. Cook (1966) regarded the neurons of buccal ganglia of *S. putris* as "possible neurosecretory cells." In *Achatina fulica* these neurons of buccal ganglia are considered to be "probable neurosecretory cells." Herlant-Meewis and Van Mol (1959) and Van Mol (1962) found neurosecretory cells in the buccal ganglion of *Arion rufus* and *Arion subfuscus*. The nsm could be traced to the n. pharyngealis secundus.

The neurosecretory mesocerebral cells in *Achatina fulica* have axons filled up with neurosecretory material. The medially situated cells send axons towards the medial border of the mesocerebrum and the anterior surface of the anterior commissure (Fig. 9). These areas are intensely fuchsinophilic and also stainable by Sudan Black B or Luxol Fast blue. This "mesocerebral-dorsal body" interface in *Achatina fulica* can be compared to the "neurosecretory neuropil" described by Simpson *et al.* (1966b) in a basommatophoran pulmonate gastropod, *H. tenue*. By ultrastructural observations they contented that the axons associated with mediodorsal body arise from neurons of the cerebral ganglion. The anterior and posterior surfaces of the cerebral commissure in *A. fulica* can functionate as a cerebral neurohaemal area. The fuchsinophilic axons of some medially situated metacerebral cells end at the posterior surface of the cerebral commissure. This posterior neurohaemal area may control the posteriorly situated part of the dorsal body. The branching twigs of the commissural nerves could be found amidst the dorsal body cells.



At the cerebral commissure neurosecretory mesocerebral cells situated more laterally had axons which joined with the similar axons from the opposite side. Similar cells situated postero-laterally had axonal bundles which course through the metacerebrum to form the nervus arteriae cerebialis which ends in the wall of the cerebral artery (Fig. 9). This is similar to the axonal migration of nsm as described by Herlant-Meewis and Van Mol (1959), Van Mol (1960, 1967). Kuhlmann (1963) observed three groups of cerebral paraldehyde fuchsin positive cells transporting nsm along the axons to the nervus arteriae cerebialis in several Helicidae. Some fibres also go to the cerebral commissural nerves. For further references the work of Van Mol (1967) may be consulted. Some mesocerebral and metacerebral cells were also seen to send short axons directly to the intracerebral blood vessels on the wall of which they arborised (Fig. 9).

In the visceral ganglion of *Achatina fulica* neurosecretory cells could be found. Some neurosecretory droplets were noted in the central neuropil. Nsm was also found amongst the capsular dorsal body-like cells. The neurosecretory material could influence the functioning of these cellular groups. The medial face of the ganglion could serve as a neurohaemal area. The connectives and nerves arising from this ganglion, did not show the presence of neurosecretory material. However, Simpson *et al.* (1966b) could find axons loaded with paraldehyde fuchsin—staining material from visceral ganglion into the periphery of the nervus intestinalis in its proximal portion and also on the anterior aorta. Cook (1966) found the visceral complex of *Succinea putris* to contain probable neurosecretory phloxinophilic neurons on the right side. They removed the nsm through the n. muscoli retractoris pharyngealis, the n. pallialis dexter, and the n. aortae. On the left side of the visceral ganglion the phloxinophilic cell group could be identified but the route of transport of nsm could not be established well. Chrome—haematoxylin—positive cell—group could be identified on the right side of the visceral complex. Similar group of cells could be found in each pleural ganglion portion of the visceral complex.

The impracticability of total extirpation of the dorsal body in *Achatina fulica* is stressed in this investigation as this is not an easily removable compact body. Incomplete surgical injury or injury by cauterization can be achieved. Moreover, this procedure always leads to more or less damage of the underlying mesocerebral neurosecretory cells. Changes in the mesocerebral cells and the cells of the dorsal body have been described. The neighbouring uninjured mesocerebral cells showed evidences of injury stimulus, with depletion of nsm and axonal migration to neurohaemal areas were well documented. Injured cells lost tinctorial affinity and there was glial proliferation. Connective tissue proliferation was noted in the injured dorsal body. The cells of the dorsal body nearer to the proliferated connective tissue, were hypertrophic with abundant neurosecretory material surrounding the cells. Similar changes have been noted by Joosse (1964) in the mediodorsal cells and cells of the dorsal bodies of *Lymnaea stagnalis*. In conclusion, he stated "Thus on the one hand the extirpation experiments do not allow further conclusions



as regards the relation of the Gomori-positive dorsal cells and the reproductive phenomena. On the other hand, such a relation is suggested as far as the dorsal bodies are concerned. These bodies which are considered to be endocrine organs, probably have a role in the processes of ovulation and oviposition."

### SUMMARY

The dorsal body of *Achatina fulica* has been described. The cellular arrangement of the dorsal body alters in different seasons of the year. In the months of July and August the cells of the dorsal bodies show alveolar and tubular arrangement. In March and April these cells take up a compact nature and the alveolar and tubular arrangement is lost. The cells of the dorsal body may produce some reproductive hormone/s. Similar cells could also be found in the capsular walls of the visceral ganglionic masses. Neurosecretory material had an access to both the situations.

Neurosecretory cells could be observed in the buccal, cerebral and visceral ganglia of *Achatina fulica*. In the buccal ganglion, the neurohaemal area could be in the posteroventral aspect. The neurons of buccal ganglion are considered to be "probable neurosecretory cells." The mesocerebral neurosecretory cells discharge the nsm at the junctional place called the *mesocerebral-dorsal body interface* on the medial side of the mesocerebrum. The neurohaemal areas include the anterior and posterior surface of the cerebral commissure, and the distribution of commissural nerves in the dorsal body. More laterally situated mesocerebral cells send their axons towards the commissure to join similar axons from mesocerebral cells of the opposite side. Posterolateral cells send axonal bundles to form nerve to the wall of the cerebral artery. Short axons from some meso and metacerebral cells can be found to end on the intracerebral blood vessels. Neurosecretory cells in the visceral ganglion have been noted in *Achatina fulica*. The discharge pathways have been described.

Response of the mesocerebral cells and the cells of the dorsal body of *Achatina fulica* has been described after surgical injury.

### LEGENDS TO FIGURES

Fig. 1(A). Mesocerebral cells of *Achatina fulica* with dorsal body. Aldehyde fuchsin stain X50.

Fig. 1(B). Dorsal body cells. Aldehyde fuchsin stain with counter stain. X215.

Fig. 2(A). Dorsal body—like cells in between visceral ganglionic masses. Aldehyde fuchsin stain with counter stain. X215.

Fig. 2(B). Dorsal body—like cells in between ganglionic masses. Aldehyde fuchsin stain with counter-stain. X215.

Fig. 3. Neurosecretory cells in buccal ganglion. Kluver-Barrera stain. X50.

Fig. 4(A). Mesocerebral neurosecretory cells. Aldehyde fuchsin stain with counterstain. X215.

Fig. 4(B). Mesocerebral neurosecretory cells. The dorsal body cells are also noted. Aldehyde fuchsin stain with counterstain. X50.

Fig. 4(C). Mesocerebral neurosecretory cells and a few cells are also noted in the neuropil.



Aldehyde fuchsin stain with counterstain. X50.

Fig. 5. The neurosecretory neuropil area in *A. fulica*. Aldehyde fuchsin stain with counterstain. X50.

Fig. 6. Neurosecretory neuropil area at posterior and posteromedial part of cerebral commissure.

Aldehyde fuchsin stain with counterstain. X50.

Fig. 7. Neurosecretion bearing nerve at the wall of the cerebral artery.

Crossman's stain.

Fig. 8(A). Visceral ganglion showing neurosecretory cells with axonal migration of neurosecretion. Luxol Fast Blue positive material in neuropil. Kluver-Barrera stain. X50.

Fig. 8(B). Visceral ganglion showing neurosecretory cells. Kluver-Barrera stain. X50.

Fig. 9. Diagram showing distribution of neurosecretory pathways and their end-stations in *Achatina Fulica*.

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## Chapter 2

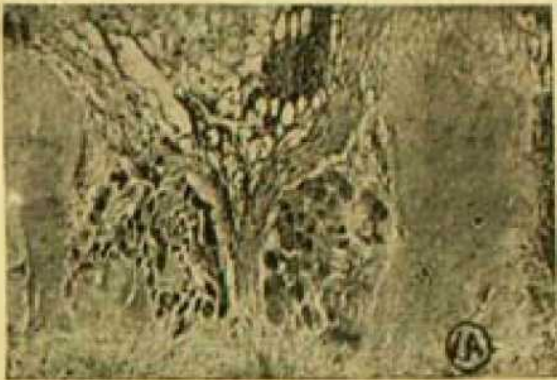


Fig. 1A



Fig. 2A



Fig. 3

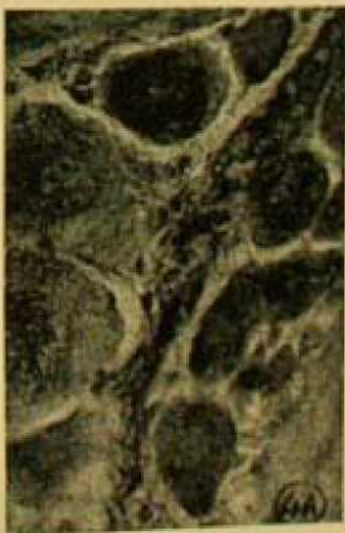


Fig. 4A

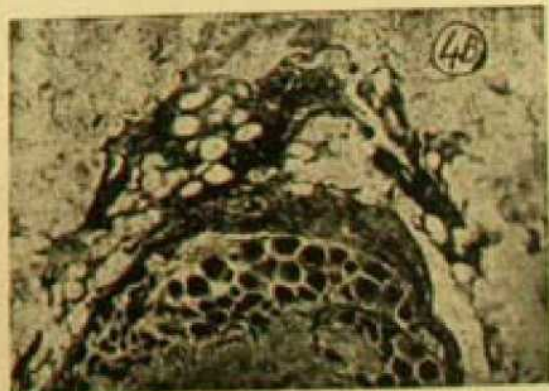


Fig. 4B





Fig. 6



Fig. 7



Fig. 8A

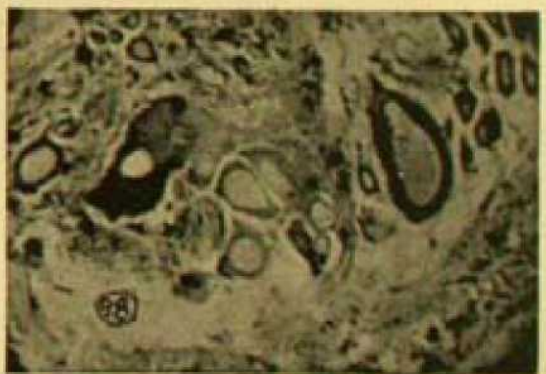
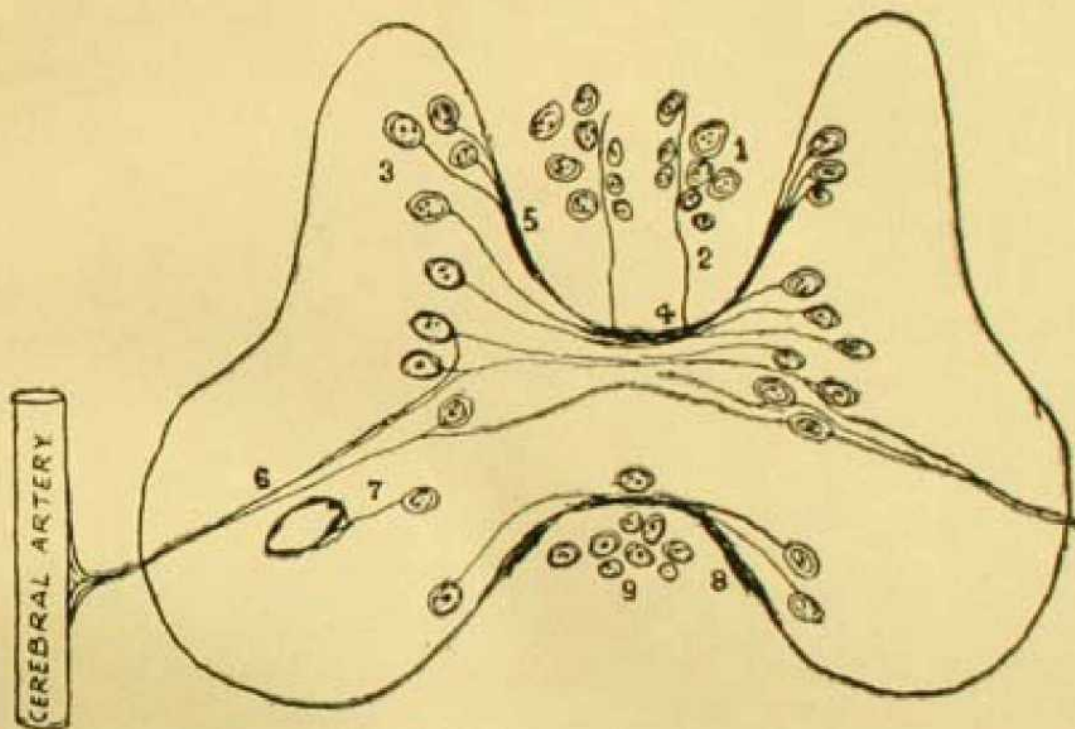


Fig. 8B



716.9

NEUROSECRETORY CELL GROUPS IN THE CEREBRAL GANGLION  
OF ACHATINA FULICA SHOWING TERMINATIONS OF AXONS IN  
NEUROHAEMAL AREAS



1. CELLS OF DORSAL BODY.
2. COMMISSURAL NERVE.
3. MESOCEREBRAL NEUROSECRETORY CELLS.
4. NEUROHAEMAL AREA AT ANTERIOR SURFACE OF CEREBRAL  
COMMISSURE
5. NEUROHAEMAL AREA BETWEEN MESOCEREBRAL CELLS AND  
DORSAL BODY CELLS.
6. AXONAL FIBRES FROM MESOCEREBRAL CELLS TO THE WALL OF  
CEREBRAL ARTERY
7. DIRECT DISCHARGE PATH TO INTRACEREBRAL BLOOD VESSEL.
8. POSTERIOR NEUROHAEMAL AREA.
9. POSTERIOR GROUP OF DORSAL BODY CELLS.





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## CHAPTER—3

### THE NEUROSECRETORY CELLS IN PALAEMON CARCINUS (1958)

The X-organ in the eye-stalk of the crustaceans was described for the first time by Hanstrom (1931). Subsequently many observers described the detailed study of it. (Hanstrom, 1933 ; 1934 *a, b* ; 1935 ; 1937 *a, b* ; 1941 ; 1947 ; 1949 ; 1953 ; Amar, 1950 ; Gabe, 1952 ; Bliss, 1951 ; Bliss and Welsh, 1952 ; Carlisle and Passano, 1953 ; Enami, 1949, 1951, 1954 ; Passano, 1951, 1952, 1953). The neurosecretory substance produced by the cells of the X-organs is transported along the axons of the cells into the sinus gland described by Hanstrom in 1931. In the sinus gland the substance was accumulated and discharged into the circulation in the nearby blood sinus. The X-organ-sinus gland complex can be compared to the pars intercerebralis—corpus cardiacum complex of insects and hypothalamo-hypophyseal complex in vertebrates. The neurosecretory centres are located in the brain, eye-stalk and thoracic ganglion. Enami (1951) studied the neurosecretory activity in the Japanese fresh water crab *Sesarma haematocheir*. Three types of neurosecretory cells were described in this species. Carlisle and Passano (1953) found at least three types of cells in the X-organ in most forms. They divided the X-organ into two parts—the pars distalis X-organi and the pars ganglinaris X-organi. These two parts being connected by a nerve which is known as connexio X-organi. The pars distalis of the X-organ acts as a storage centre like the sinus gland. When the sinus gland is removed, there is accumulation of neurosecretory material at the proximal stump (Passano, 1953). A new sinus gland like structure is regenerated as has been shown by Enami (1954). Bliss and Welsh (1952).

The neurosecretory substance is responsible for colour change, retinal pigment migration, molting and growth, reproduction, metabolism and others.

Passano (1952, 1953) first described the detailed study of neurosecretory material in the living tissue. In *Sesarma reticulatum*, he described "spheres" or "spheroid systems" in living neurosecretory cells. These highly refractile granules were about  $0.3\mu$  in diameter and these were found surrounding an optically empty central area. At times they coalesced to form big droplets. These were found in cell bodies, axons and sinus glands.

#### *Investigations in Palaemon carcinus :*

##### STUDIES INCLUDE :

- (a) Dark field illumination of the neurosecretory centres.
- (b) Staining of the sections by Gomori's chrome-alum-haematoxylin-phloxine method after fixation in Bouin's fluid.
- (c) Aniline blue method.



## Chapter—3

### The Neurosecretory cells in *Palaemon Carcinus*.



Fig. 1

Fig. 1. Dark-field illumination study. Highly refractile granules are seen in the neurosecretory cell around an optically empty area.



Fig. 2

Fig. 2. Neurosecretory cell groups in the brain of *Palaemon carcinus* in different stages of activity (CAHP Stain of Gomori).



Neurosecretory cells are found in the brain, eye-stalk and thoracic ganglion. Under dark field illumination, highly refractile substance is found in the cells, axons and sinus gland. The neurosecretory substance is stainable by Aniline blue or by Gomori's method. Majority of the neurosecretory substance takes up haematoxylin component of the Gomori's colour but some takes up the phloxine component. The X-organ of the *Palaemon carcinus* is composed of big monopolar cells, and rounded cells found in groups. Cells of the syncytial pattern are also found. The X-organ can be divided into a pars distalis part which is a storage centre and a pars ganglionaris part which is a productive one. These two parts are joined together by a nerve. The sinus gland is another storage centre.

When the sinus gland is removed, there is accumulation of neurosecretory substance in the proximal stump.

One of the controls of the neurosecretory centres in the brain is by light acting directly on the centres penetrating through the more or less transparent structures above the brain.

Watery extracts of eye-stalk, brain and thoracic ganglion give rise to depletion of sudanophilic substance in the suprarenal gland of *Bufo melanostictus*. This is an example of invertebrate neurosecretion acting on vertebrate target organ.

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## CHAPTER—4

### HYPOTHALAMO-PITUITARY-ADRENAL-AXIS AND ITS MATURATION (1958)

#### (A) NEUROSECRETION :

In the hypothalamus of many types of vertebrates and man, cells with cytological characteristics of glandular structures were described by E. Scharrer (1928 and afterwards), Scharrer and Gaupp (1933), Scharrer and Scharrer (1954), Scharrer and Wittenstein (1952). The presence of posterior lobe hormones in the hypothalamus was shown by Abel (1924), Sato (1929), Trendelenberg (1928), and Melville and Hare (1945). The posterior lobe hormones were shown to be formed by the neurosecretory cells of the hypothalamus by Hild and Zetler (1951, 1952, 1953),—Ortmann (1950, 1951), Bargmann (1949, 1954), Hild (1954) and others. The posterior lobe hormones are transported by axoplasm current along the tractus supraoptico-hypophyseus to the neurohypophysis where they are stored and released on requirement. The posterior lobe is thus an organ of storage for the hormones. There are two other views regarding the origin of the posterior lobe hormones.

- (a) They are produced by degeneration of the basophilic cells which migrated from the adnohypophysis to the neurohypophysis (Herring, 1915 ; Cushing, 1933 and others).
- (b) The hormones are formed by specific gland cells—the pituitocytes in the posterior pituitary (De Lawder *et al.* 1943 ; Gersh, 1937, 1939 ; Fisher, 1937 ; Ranson, Fisher and Ingram, 1938).

Hild (1954) solved this problem by *in vitro* experiments. He found that posterior pituitary tissue *in vitro* and in the living organism was not able to form hormones. When the posterior pituitary tissue is taken for culture, some amount of the hormones is stored in it and it is liberated into the culture medium where it is present for several days. If the posterior pituitary is taken from a dehydrated animal, hormonal activity in the culture medium is never present. He further says that he could not find any difference between the glia cells of the posterior pituitary and ordinary glia in the brain. From these experiments it can be derived that the posterior pituitary hormones are not produced in the posterior pituitary but they are produced in the hypothalamus and the posterior pituitary is simply a storage organ. There is a close parallelism between the amount of neurosecretory material and the hypothalamic and neurohypophyseal hormones in dog, ox, pig and man (Hild and Zetler, 1952). Highest concentration was found in the dog and lowest in man. Dehydration gives rise to depletion of the neurosecretory substance but when water is allowed there is re-accumulation of the Gomori-stainable material. In these ex-



perimental conditions a parallelism is found between ADH and neurosecretory substance. Hild and Zetler (1953) studied the problem further by stalk section in dogs where it was found that neurosecretory substance and hormonal content diminished distal to the stalk section but in the proximal side there was an increase above control levels. An accumulation of neurosecretory substance proximal to the area of stalk section has been observed in different vertebrates by many (Stutinsky, 1951, 1954; Hild, 1951; Mazzi, 1954; Benoit and Assenmacher, 1954; Scharrer and Wittenstein, 1953; Roy, 1957). Experiments on invertebrates also corroborate the above (Passano, 1951; Roy, 1958; and others). Increased activities in the proximal stump and even in areas more proximally have been observed by many (Lloyd and Pierog, 1955; Barnett, 1954; and Billenstien and Leveque, 1955). Hild (1956) found by time lapse microcinematography (phase contrast) of axon processes of neurosecretory neurons *in vitro* (perfusion chamber technique) that there was evidence of peripherally directed transportation of substances within the axoplasm of these cells.

#### HORMONAL ACTIVITY IN THE EXPLANTS OF SUPRAOPTIC AND PARAVENTRICULAR NUCLEI

Borghese (1954) could not find intracytoplasmic colloid granules or masses in the cultures. The reason is that the *number of nerve cells* migrating was small and thus the amount of the material expelled was less which could not be detected by the relatively crude bioassay methods. Hild (1956) was of the same opinion. Though the nuclei cultured *in vitro* do not discharge (?) any hormone but a peculiar feature has been studied by Guillemin (1956). In tissue cultures of the pituitary, ACTH activity was never found after more than four days *in vitro*. When fragments of posterior hypothalamus or of the median eminence was cultured with the pituitary tissues from day fifteen to nineteen or twenty-two to twenty-six, ACTH activity was again found. Anterior hypothalamus had no ACTH releasing activity in the pituitary explants. But in organ culture of pituitary both anterior and posterior hypothalamus of bovine origin had stimulating effect on ACTH secretion. From the above findings Guillemin questioned whether we should consider that the two different regions (anterior and posterior) of the hypothalamus may be concerned with ACTH release by different mechanism or it might be that the active hypophysiotropic substances originated in particular nuclei may pass to adjacent areas.

Lastly the question remains to be answered is that whether the neurosecretory substance stained by Gomori's CAHP stain is actually the hormone itself or merely a carrier for the hormone. The neurosecretory granules are easily dissolved in acetone or alcohol and they had no hormonal effects; but the tissue thus freed of the granules had hormonal activities. It proves that the stainable material is a carrier for the hormones. It has been previously mentioned that there is a close parallelism between Gomori-tingible material and hormonal activity. Adams and Sloper (1955) developed a method by which they stain only the neurosecretory material. The technique involved reduction of ferric-ferricyanide and



it reacted only with high concentration of cystine. According to them the tingible neurosecretory substance is the hormone itself.

### (B) NEUROSECRETION IN FOETAL AND POSTNATAL LIFE

#### (a) *In the tadpole Rana agilis* :

Mazzi (1954) found the neurosecretory substance first in the neurohypophysis.

I have personally found in the tadpole *Bufo melanostictus* the neurosecretory substance in the hypothalamic cells (Preoptic nucleus).

(b) *In Chicken*—Wingstrand (1953) found the early appearance of the neurosecretory substance in the chicken embryo. No histological evidence of the substance was found until 13th or 14th day. Mosier (1955) found the substance in the neurones of the supraoptic region of the chicken embryo after 97 hours' incubation.

- (1) In the axons at five days' incubation.
- (2) In the infundibulum by sixth day.
- (3) Increased concentration in the infundibulum thereafter throughout the embryonic life.

(c) *In the Rat*—Dawson (1953) found the substance to be present in the hypophysis of the newborn rat (aldehyde fuchsin method).

The substance was present in the hypothalamic nuclei by the end of the 2nd day. It was present in the fibre tracts on the sixth day. CAHP positive substance was present in the neurohypophysis on the sixth day.

(d) *In the Dog*—Bargmann (1949) could not find neurosecretory substance in the hypophysis of the newborn dog. In the 25 day-old dog the neurosecretory substance first appeared in the ganglion cells of the hypothalamus. But Scharrer (1954) could find neurosecretory substance in the foetus of the dog. The substance first appeared in the posterior lobe of the hypophysis and then in the hypothalamic nuclei.

(e) Rodeck and Caesar (1956) studied the neurosecretory system in rats, guineapigs, dogs and human foetuses, newborn infants and children. They concluded that at birth the system is not well differentiated.

(f) *Cow Embryo*—Kivalo and Talanti (1957) demonstrated the neurosecretory substance in the hypothalamic cells and in the neurohypophysis at the gestational age of 3 months (Aldehyde fuchsin method). After that the material gradually increased. The postnatal quantity of the material was greater than the prenatal one.

(g) *Human Foetus*—At the 20th week of gestation, the neurosecretory substance was present in the hypothalamic cells. The substance was present in the posterior lobe at the twenty third week of gestation (Benirschke and Mackay, 1953).

(h) *Goat Embryo*—I have studied the materials by CAHP stain of Gomori.



*Hypothalamus*—Towards the end of the early part of the gestational life, the neurosecretory substance was present in the magnocellular nucleic cells. Towards the end of the gestational life the substance is definitely increased and in the postnatal life it is more so.

*Neurohypophysis*—The neurosecretory substance appeared in the neurohypophysis at the same time it was found in the hypothalamus and neural stalk. Its intensity was increased towards the end of gestational life. In the postnatal period the substance was found in the perivascular spaces.

From the above it is seen that most of the authors could detect an early appearance of the neurosecretory substance in the system of different vertebrates.

### (C) IMPORTANCE OF THE NEUROSECRETION IN THE ACTIVATION OF THE PITUITARY-ADRENAL AXIS

de Groot and Harris (1950) suggested that some neural mechanism in the hypothalamus maintained and regulated the secretion of ACTH by the anterior pituitary. Hypothalamic nerve fibres liberated some chemical transmitter into the hypophysial portal vessels. The substance was then carried to the anterior pituitary gland to activate it. Since then, works have been done by many in different types of animals to study the role of hypothalamus in pituitary ACTH activation by stimulation and lesion experiments of the hypothalamus, pituitary stalk section and pituitary transplants placed in the anterior chamber of the eye of animals. Majority of the workers agree regarding the importance of the hypothalamus in this respect. (The detailed account of such experiments and results is not presented here). I have done stalk section experiments and measures taken to prevent vascular regeneration in guineapigs and studied the response of pituitary-adrenal-axis to the stress of fractures and intramedullary pinnings after fractures and burns where increased adrenocortical activation was found. The types of stresses used by me were severe. Using different types of stress others could find no adrenocortical response. The explanation being that in the severe types of stresses sufficient neurosecretory substance comes in the general circulation which then can activate the anterior pituitary ACTH secretion. Or it may be that histamine liberated in the types of stress used by me is responsible for the increased activation of the pituitary-adrenal-axis. Harris (personal communication) mentioned about the importance of histamine in this respect. This can also be explained either by Sayers' peripheral humoral concept or by Long's adrenaline hypothesis.

Harris (1955) questioned whether the hypothalamus played a part in regulating the detailed pattern of anterior pituitary secretion. This question can be answered from the work of Harris and Jacobsohn (1952). It was found that the anterior pituitary tissue from a *male rat*, when placed under the stalk of a hypophysectomized *female rat* showed normal female



reproductive activity, e.g. rhythm of oestrous cycles, pregnancy and lactogenesis. Harris concluded that "the hypothalamus supplies not only a general stimulus to anterior pituitary function, but also sets the pattern of this function."

Next question he asked whether there was as many humoral mechanisms involved as there were hormones. This question cannot be answered definitely until there is a good technique for measuring the anterior pituitary hormones directly from the pituitary venous effluent.

He suggested that the same humoral stimulus at different thresholds could discharge the different anterior pituitary hormones.

Finally he mentions "If different cell types are dependent on a different humoral stimulus, and if the liberation of humoral stimuli are regulated by different nerve fibre tracts in the hypothalamus, a correlation might be drawn between medial and lateral nerve tracts in the median eminence, medial and lateral trunks of the portal vessel in the pituitary stalk and a medial and lateral grouping of cell types in the pars distalis. There are good grounds for believing that the various portal trunks supply different areas in the pars distalis."

#### (D) ACTIVITY IN THE PITUITARY-ADRENAL AXIS IN NEWBORN ANIMALS AND INFANTS

(a) *Rats*—Injection of adrenaline in the newborn and young rats did not give rise to ACTH discharge until the 8th day of life and cold exposure to 5°C for 75–150 minutes was not effective until the 16th day. It may be due to the non-development of the hypothalamic nervous pathways—(Jailer, 1950).

(b) *Mice*—Eosinopenia in mice less than one day old was found after ACTH or adrenaline; but with heat stress this response could not be observed till about the 11th day of life. Failure of response to heat stimulus could be due to an interruption in the chain at a prepituitary level between the reception of heat stimulus and discharge of ACTH—(Thomson and Blount, 1954).

(c) *Dogs*—Upto twelve days after birth young dogs were completely insensitive to the injection of ACTH, the dose of which was sufficient to give rise to a fall in the blood eosinophil level in adult dogs. After twelve days, progressively greater response was achieved with smaller doses of ACTH (Morel *et al.*, 1951).

(d) *Infants*—Most full-term infants in the first 24 hours showed a fall in the total eosinophil count after epinephrine injection but in premature infants significant fall in total eosinophil count in response to epinephrine did not occur before 9th day of age. Results with ACTH injection showed that there was no lack of sensitivity of the target organs—(Jailer *et al.* 1951). But Venning (1951) could find no difference in urinary excretion of glucocorticoids in between full-term and premature infants.



Increased excretion of glucocorticoids was found after the second week of life. In the new-born infant, there was a fall in the eosinophil count and a rise in the urinary corticoids and 17-ketosteroids, after injection of ACTH. Venning thought that the adrenal gland was less responsive to ACTH at birth than in the second week of life. Atelectasis in the new-born premature infant gave rise to increased excretion of corticoids.

(e) *Guineapigs and Infants :*

In new born guineapigs increased adrenocortical activity has been observed by me after the stressing procedures of fractures and burns. In new-born full term infants, surgical intervention for imperforate anus (colostomy) or for congenital obstruction in the urethra by septa, there is a fall in the total eosinophil count proving that there is increased adrenocortical activity in these patients. Similarly new-born infants having birth fractures show increased adrenocortical activity.

From the above observations it is seen that in the new-born animals or infants increased adrenocortical activity is found and that depends on the type of stress.

Rickham (1957) studied the metabolic response to major surgical procedures between infants to neonatal surgery. There is a clear difference in the response in patients of a few days old and those over two weeks old. Drop in the eosinophil count was noted after operations. The smallest infant, case 6 (Esther 0) had the additional disadvantage of being six weeks premature. The patient had duodenal obstruction. The eosinophil count was very low throughout the period of study and it was never 100 per cubic m.m. A slight rise on the fourth and *last day* was found. The obvious metabolic difference in between neonates and older children lies in the potassium metabolism. Rickham mentions "It is tempting to correlate our findings with the pituitary-adrenal response which has been observed in neonates. It also appears reasonable to suggest that the metabolic peculiarities observed in new-born infants are, in part at any rate, responsible for the extraordinary resistance that such infants have to prolonged surgical interference—resistance which, as clinical experience shows, lasts for only a few weeks after birth and passes off when the neonatal metabolic response changes over to the adult pattern." The cause of this resistance may be due to physiological processes in the foetus or due to the presence of maternal hormones in the foetus.

Stoner *et al.* (1953) found the steroid content of the adrenal gland to be low in the neonatal period and the adrenals of young infants do not respond to systemic disease. They thought that as the new-born infants stand operative interferences better, it might be that the infants received adequate supplies of the cortical hormones from their mother. Lanman (1953a, b) found that in proportion to surface area new-born infants excreted more steroids than adults after ACTH stimulation and that the adrenal function was not impaired in the premature and full-term babies. After ACTH injection, the new-born infants excreted more sodium instead of retaining it.



## (E) STUDY OF THE FOETAL PITUITARY-ADRENAL AXIS

Alfred Jost in 1953 discussed the problem of foetal endocrinology nicely. The types of experiments by which the study of foetal pituitary-adrenal axis has been done, are as follows :

- (i) In mice either a subtotal or a total destruction of the hypophysis on day 13 by X-ray gave rise to atrophy of the adrenal cortex at birth (Raynaud, 1943 ; Raynaud and Frilley, 1950).
- (ii) In decapitated rat (Domm and Leroy, 1951 ; Jost, 1951 ; Wells, 1947 ; 1948) and rabbit foetuses (Jost, 1948) there was adreno-cortical atrophy.
- (iii) Adrenals in decapitated foetuses did not show atrophy when corticotrophic hormone was administered (Jost, 1951 ; Wells, 1948).
- (iv) Unilateral adrenalectomy leads to hypertrophy of the other adrenal. Kitchell and Wells (1952) could prevent the compensatory hypertrophy in the foetal adrenal after unilateral adrenalectomy when compound E was given.

Jost (1953) says "The observations reported upto now give rather good evidences for production of corticotrophin by the fetal hypophysis during the last part of fetal development.....Finally the question of the dependence upon hypophyseal stimulation of the initiation of adrenal physiological activity remains yet to be studied."

## (E) STUDY OF THE PITUITARY ADRENAL AXIS IN ANENCEPHALICS

Boulgakow (1926) stresses the importance of the influence of the central nervous system during foetal life. In the anencephalus holocarcidicus there is arrest of development of all parts of the organism except the skeletal system as a result of the absence of this influence. In the suprarenals there is zona glomerulosa, fasciculata and reticularis slightly degenerated but in his drawing (Fig. 5) the suprarenals seem to be smaller in size. He says "although it is true that many organs are present, as I have proved microscopically, all the tissues and organs are in an embryonic stage and they are only enlarged in size without further development."

The foetal adrenal is large because of the presence of the "foetal cortex" which rapidly involutes after birth. The "permanent cortex" is small in the foetal stage and it proliferates during the last ten weeks of gestation (Keene and Heward, 1927). The true cortex and foetal cortex are generally considered to develop from a common origin. They differentiate from the cells forming the original mass in the 10 m.m. embryo, but Keene and Heward (1927) suggest that this original mass gives rise to only foetal cortex, the true cortex arises from the cap of cells which is seen at a later period.

In the anencephalics the adrenals are small. There is early atrophy of the foetal cortex in cases of anencephaly (Keene and Heward, 1927 ;



Elliott and Armour, 1911 ; and Meyer, 1921). In an anencephalous embryo of 25 m.m. studied by Keene and Hewer (1927) the suprarenal was well developed and there was usual mass of foetal cortex and a narrow rim of developing true cortex. Their examination of anencephalous suprarenal (24-26 weeks) revealed considerable amount of degenerating foetal cortex and the gland was extremely small. In the case of cerebromeningocoele (4(b)) the brain was mostly degenerated and the foetal cortex of the suprarenal gland was very small whereas in the case (4(a)) where the cerebellum only was involved in the hernia the suprarenal gland was normal for its age. They further mention "That the acephalous state is constantly associated with certain abnormal appearances of the suprarenal gland is indisputable, but the nature of the relationship is obscure." Mayer (1912) observed normal sized adrenals in anencephalics of the second and fifth month of gestation but for unknown causes the adrenal atrophied after fifth month of gestation. Angevine (1938) found larger adrenal glands in smaller anencephalics. Benirschke (1956) found normal foetal zone in small anencephalics whereas this foetal zone atrophied after approximately 20 weeks of gestation.

#### LIPIDE CONTENT AND DISTRIBUTION IN THE FOETAL ADRENAL

Keene and Hewer (1927) observed the lipid granules in the 16-week embryo, in a few large migratory cells. The cells migrate through the cortex and rest in the central part of the gland. By 22 weeks first trace of lipid is seen in the cells of the true cortex as very fine small granules.

*By the 24th week* the cells of the foetal cortex also contain very minute lipid granules. During later months of foetal life the lipid is increased in the true cortex. At full term the foetal cortex contains no lipid but the cells of the true cortex show increased lipid stain. After birth lipid is present in the developing new cortex. The degenerating foetal cortex does not show any fat.

Hett (1925) observed the earliest lipide in a 23 mm.. human embryo. Noel and Pigeaud (1931) found the typical lipid filled spongiocytes in the cortical anlage at the age of 2 months 23 days. These authors ascribed the mitochondria as the lipid builder. Velican (1948) described the lipid granules in 6 month old foetus.

#### DIFFERENTIATION OF CORTICAL CELLS AND STARTING OF THE SECRETORY ACTIVITY IN THE ADRENAL CORTEX

Cellular differentiation in the suprarenal cortex in the foetus is early. In the embryo of 2 months and 23 days Noel and Pigeaud (1931) described three different cell types by staining with iron haematoxylin.

(a) Homogeneous cells, (b) Vacuolar cells filled up with lipid and (c) transition form and it is said that the secretion cycle starts. According to Kolmer (1918) the cytological differentiation in the cortex starts at the age of 3 to 4 months. Hett (1925) thought that the secretory activity starts



at the age of 6 months. Bachmann (1954) is also of the opinion that the secretory activity starts early in the foetal adrenal cortex.

Adrenalin is an important agent for the increased adrenocortical activity. The following findings are from Keene and Hewer (1927). The gland extract of a twelve-week embryo had a trace of adrenin. At 16 weeks adrenin was definitely present. At 18 weeks and onwards adrenin was found to be present in good amount. Chromaphil reaction was never found before 22 weeks of foetal life. Dietrich and Siegmund (1926) found the positive chromaphil reaction in the suprarenal medulla at the foetal age of 3 to 4 months. Velican (1948) found the same at the age of 6 months.

From the above it is seen that the activity in both the cortex and medulla starts early in foetal life.

#### CAVITIES, LUMINA AND FOLLICULAR STRUCTURE IN THE FOETAL SUPRARENAL GLAND

Starting of the activity of the suprarenal cortex early in foetal life can also be understood from the above type of changes in the adrenal cortex. Keene and Hewer (1927) speak of the arrangement of cortical cells around central space in an 11-week embryo. This appearance was specially marked in 16-week embryos and the "vesicles" sometimes contained a basophil substance that appeared like a coagulated fluid. In the later months of foetal life this type of vesicular arrangement of the outer zone is less marked but occasionally this is present even after birth. Such types of cavities, lumina or follicular structure in the outer zone of the adrenal cortex in foetal life have been described by Hett (1925).

#### HORMONES IN THE FOETAL ADRENAL CORTEX

Benirschke, Bloch and Hertig (1956) made chemical analysis of extracts of foetal adrenal glands and found the presence of weak androgenic steroids and a sodium retaining factor. These steroids were high in smaller foetuses and were approximately equal in the male and female foetus. In older foetuses the glands contained small amounts of 17-hydroxycorticosterone. They thought that  $C_{19}$  steroidogenesis was limited to the foetal cortex and the  $C_{21}$  steroidogenesis was carried out by the definitive cortex. Staemmler (1953) observed a gradual increase of the  $C_{21}$  steroids in the foetal adrenal gland after the fifth month of gestational age.  $C_{21}$  steroids extracted per unit of adrenal from four anencephalic monsters were either equal to or higher than that found in normal foetuses of the same gestational age (Kloos and Staemmler, 1953). There was atrophy of the foetal zone and the adrenal gland was composed of practically by the definitive cortex. Bloch *et al* (1956) suggests "That the early fetal adrenal cortex synthesizes primarily weak androgenic steroids and a sodium retaining factor similar to aldosterone.  $C_{19}$  steroid synthesis is considered to occur in the fetal zone with decreasing activity as fetal age advances. The hypothesis is advanced that the fetal and reticular



zones are the sites of adrenal androgen production, and the fascicular and glomerular zones, respectively the sites of glucocorticoid and mineralocorticoid synthesis, respectively."

The glucocorticoids are not found in the early foetal adrenal tissues. The causes are :

- (a) sub-minimal ACTH production in the foetal pituitary,
- (b) release of ACTH by the foetal pituitary is defective,
- (c) corticoids from mother would inhibit foetal ACTH production or release,
- (d) the fascicular zone not producing the glucocorticoid at such an early foetal age.

The control of production of  $C_{19}$  steroids from the foetal zone is by lutenizing hormone (Benirschke *et al.* 1956). In the early gestational period LH comes from the maternal chorionic gonadotrophin. After the midgestational period maternal oestrogen stimulates the foetal pituitary to produce LH. After birth oestrogen from mother is not present and so there is fall in  $C_{19}$  steroid synthesis.

Rotter (1949, 1950) says that the inner cortical part of the foetal suprarenal is under the influence of the gonadotrophic chorionic hormone of the placenta. When this falls after birth, there is a breakdown in this zone. The outer zone is developed possibly through the action of the corticotrophic hormone from the anterior pituitary. The corticotrophic hormone of the mother cannot pass through the placental barrier and come to the foetal side because possibly due to its large molecular size. That the foetal cortex can produce corticosteroid has been shown by Zander and Solth (1953). Immediately after birth in both sexes, there is a fall in corticoid excretion in urine which according to these authors corresponds with the destruction of the inner zone of the suprarenal.

#### PERSONAL OBSERVATION

##### *Neurosecretion :*

The neurosecretory substance as stained by Gomori's chrome alum haematoxylin phloxine method appears in the hypothalamus at about 20th week of gestation (Benirschke and McKay, 1953). I am personally of the same opinion as that of Benirschke and McKay (1953). In the anencephalic foetus, the neurosecretory substance does not appear in the ill-formed or destroyed hypothalamus and neurohypophysis. The ill-formed neurohypophysis in a particular case has been converted into a multicystic structure. The adenohypophysis does not show any gross maldevelopment.

*Adrenals*—The adrenals are small. When stained with sudan IV the distribution of lipide in a normal foetal suprarenal is as follows :

In the early period, the sudanophilic substance in the permanent cortex is fine and less in amount. This increases with advanced gestational age. The inner or foetal cortex contains large amount of sudanophilic substance in the early part of foetal life and as foetal age advances it is diminished. In the anencephalic foetus with advancing foetal age



the sudanophilic substance is remarkably less in the atrophied foetal zone but the substance is present in the permanent cortex. In the permanent cortex lipide loss may be found. This may be due to the stress to which these foetuses are subjected. Similar type of lipide depletion is also found in foetuses of eclamptic mothers or when they suffer from toxæmias of pregnancy. This shows that the maternal stress is also manifest in foetuses and this is of humoral in nature. In asphyxiated conditions such features are also observed.

Adrenals when stained with haematoxylin and eosin show haemorrhages in the atrophied foetal cortex in anencephalic foetus. This is a change which occurs during involution of this zone. Vacuolar, luminal and tubule formations are also found in the permanent cortex. Similar change is also found in the accessory cortex. These are all evidences of increased adrenocortical secretion. Such changes are not only found in anencephalics but in other foetal glands from stress conditions. The adrenal medulla does not show any major alteration. Gemzell *et al.* (1956) said "In general the concentration of 17-hydroxycorticosteroids in the umbilical cord of the newborn infant is about 60 per cent. of the level of the mother. But levels higher than those of the mothers are found in infant delivered after signs of foetal distress and hypoxia, and extremely low values are found in infants delivered by Caesarean section. It may therefore be suggested that the levels of plasma steroids in the mother and the foetus reflect the various stress promoting factors acting on each one in labour (Gemzell, 1954). The plasma steroid level and urinary excretion rate of corticosteroids of the mother may therefore reflect the activity of her adrenal cortex, and the plasma steroid level of the umbilical cord may reflect the activity of the foetal adrenal cortex."

The finding of normal foetal zone before 5 months of gestational age in the anencephalic foetus and the atrophy of the same after 5 months, brings the question of its dual control. In the early part of the foetal life placental chorionic gonadotrophin maintains this zone whereas in later period the LH coming from the foetal pituitary controls it, but because in the anencephalic foetus the second type of control is deficient, the maintenance of this zone is not possible.

Hypothalamic neurosecretory substance comes to the anterior pituitary via the hypophyseoportal vessels and regulates the liberation of anterior pituitary hormones. This has been proved by pituitary stalk section and hypothalamic lesion experiments and others. These are comparable to the hypothalamo-hypophyseal axis in anencephalics where the hypothalamus is at fault ; but the difference is that in the experimental animals the hypothalamus, the neurosecretory path, the hypophyseoportal vessels were alright prior to the starting of the experiments, whereas in the anencephalics these are not upto the standard. Still then in such foetuses (anencephalics) the  $C_{21}$  steroids were either equal to or higher than that found in normal foetuses of the same gestational age (Kloos and Staemmler, 1953) and also from the histological study of adrenals in anencephaly evidences of increased adrenocortical activity are found. So, when the hypothalamus is not controlling the pituitary adrenal axis, the question comes



up regarding the control of the adrenal cortex in such circumstances. This may be a *peripheral control* or the *anterior pituitary without the influence of the hypothalamus* can control the adrenal cortex. The anterior pituitary devoid of the hypothalamic control can respond to stress situation in experimental animals where the axis functionated alright prior to the experiment ; but in the anencephalic foetuses the situation is different and we are to consider whether the anterior pituitary functionates at all or not. The finding of the secretion histologically in the anterior pituitary in the anencephalic foetus leads one to think about the activity in this gland. The secretion as histologically demonstrated is very small and so its control, if at all, over the foetal adrenal cortex is very little. The other type of control is by a purely peripheral mechanism without the help of the pituitary gland. Such a type has also been considered by Okinaka *et al.* (1954). They proposed that the N. splanchnicus-adrenocortical system may play an importance role in the defense mechanism of the body. "The fact that the chemocorticoid substance is mobilized in the hypophysectomized animal either by the stimulation of the N. Splanchnicus or by the epinephrine-injection, suggests the possibility that the secretory mechanism of the adrenal cortex may be under the control of the peripheral neurohumoral mechanism besides under the ACTH through the anterior pituitary gland." Such a type of peripheral control in the foetal stage can also be thought from the following observations of Keene and Hewer (1927) :

- (a) appearance of chromaphil reaction in the adrenal medulla after 22 weeks of foetal life.
- (b) at 16 weeks adrenin was definitely present in the gland extract.
- (c) nerve cells in the semilunar ganglia acquired the adult character more quickly.
- (d) "the pre and para-aortic tissue of a 16-week embryo from the region of the suprarenal glands is seen on section to contain the semilunar ganglia, collections of haemolymphoid tissue, and other encapsulated masses (the Zuckerkandl anlage) consisting of cells arranged in groups. These cells have large faintly staining nuclei, and although the cell boundaries are rather ill defined the cells are arranged in groups and are well supplied with capillaries.....under the high power of magnification the cells of Zuckerkandl bodies at full time resemble the large cells described in the suprarenal medulla, and like them show the chromaphil reaction in both protoplasm and nucleus." The body progressively atrophies after birth.
- (e) The adrenal medulla in the anencephalic foetus is big.

A close relationship thus exists between the para-ganglion and Zuckerkandl body, adrenal medulla and adrenal cortex in the foetal condition.

#### CONCLUSION

- (1) Neurosecretion in the tadpole *Bufo melanostictus*, and in the goat embryo has been described.



(2) Stress of fracture, intramedullary pinning after fracture and burns in guineapigs with pituitary stalk sectioned and measures taken to prevent vascular regeneration shows increased adrenocortical activity.

(3) Increased adrenocortical activity in newborn guineapigs and infants is seen after stress.

(4) Neurosecretory substance appears in the hypothalamus and neurohypophysis of human foetuses after 20th week of gestation. The substance is not present in the destroyed or illformed neurohypophysis and hypothalamus.

(5) Distribution of lipide in the adrenal in foetal stage and in the anencephalics is described.

(6) Maternal stress is manifest in foetal adrenals.

(7) Foetal adrenals show changes in stress.

(8) Vacuolar, luminal and tubule formation is found in the permanent cortex and accessory cortex of the anencephalics and also in the adrenals of stressed foetuses. These are evidences of increased adreno-cortical secretion. The adrenal medulla does not show any major alteration and it is big in the anencephalic.

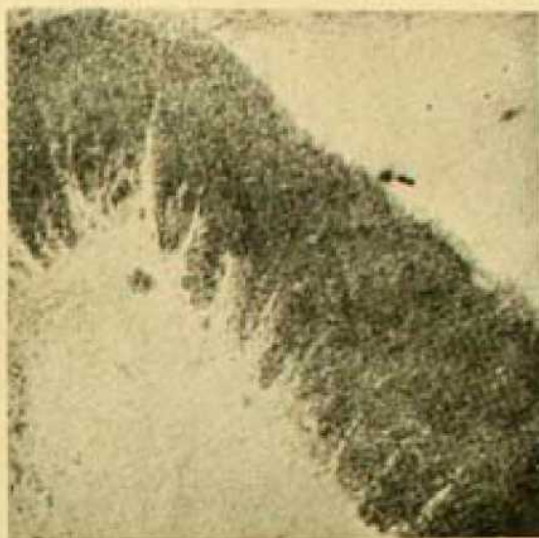
(9) Hypothalamo-hypophyseal control of the adrenals is present for normal foetuses and a peripheral control is suggested for the adrenals of the anencephalic foetuses.

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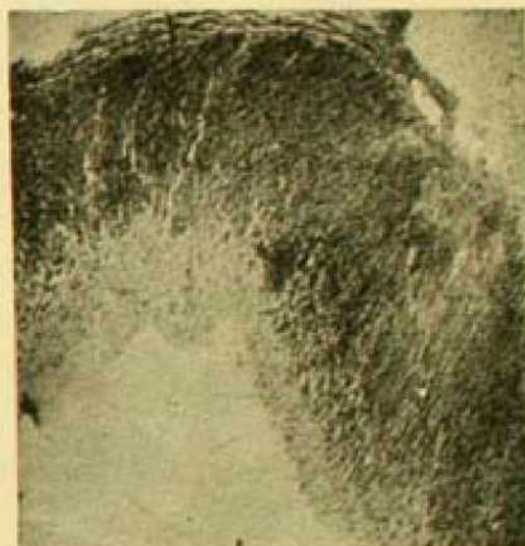
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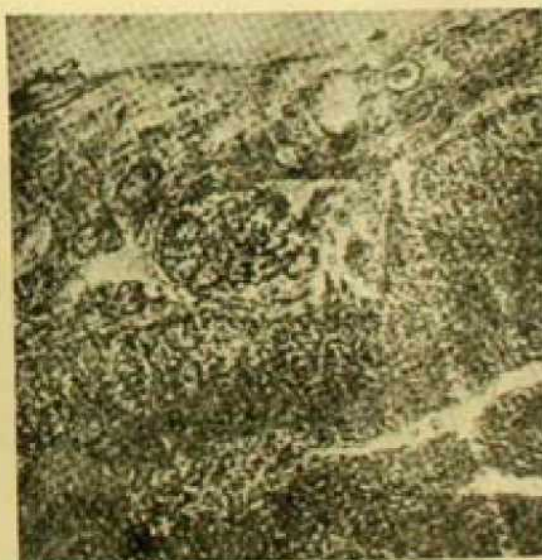
## Chapter 4



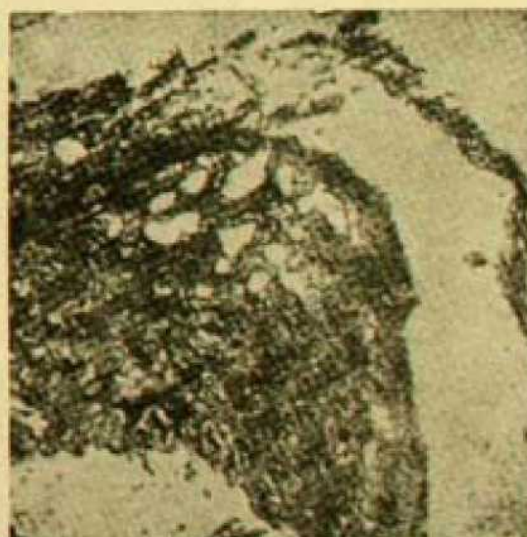
**Fig. 2**  
Adrenal gland of a new-born baby showing the distribution of lipoid substances stained by *Sudan IV*.



**Fig. 3**  
Adrenal gland of a new-born asphyxiated baby showing loss of Sudanophilic substances from the Cortical zones—*Sudan IV Stain*.



**Fig. 5**  
Adrenal gland from an anencephalic fetus showing the accessory cortex and the true cortex with cytolitic changes—there are hemorrhages in the fetal cortex which is extremely narrowed and the true cortex is much thickened—*H & E Stain*.



**Fig. 6**  
Multicystic appearance of the neural lobe of the Pituitary from an anencephalic fetus—*H & E Stain*.



## CONTROL OF THE ADRENAL CORTEX IN A NORMAL FOETUS

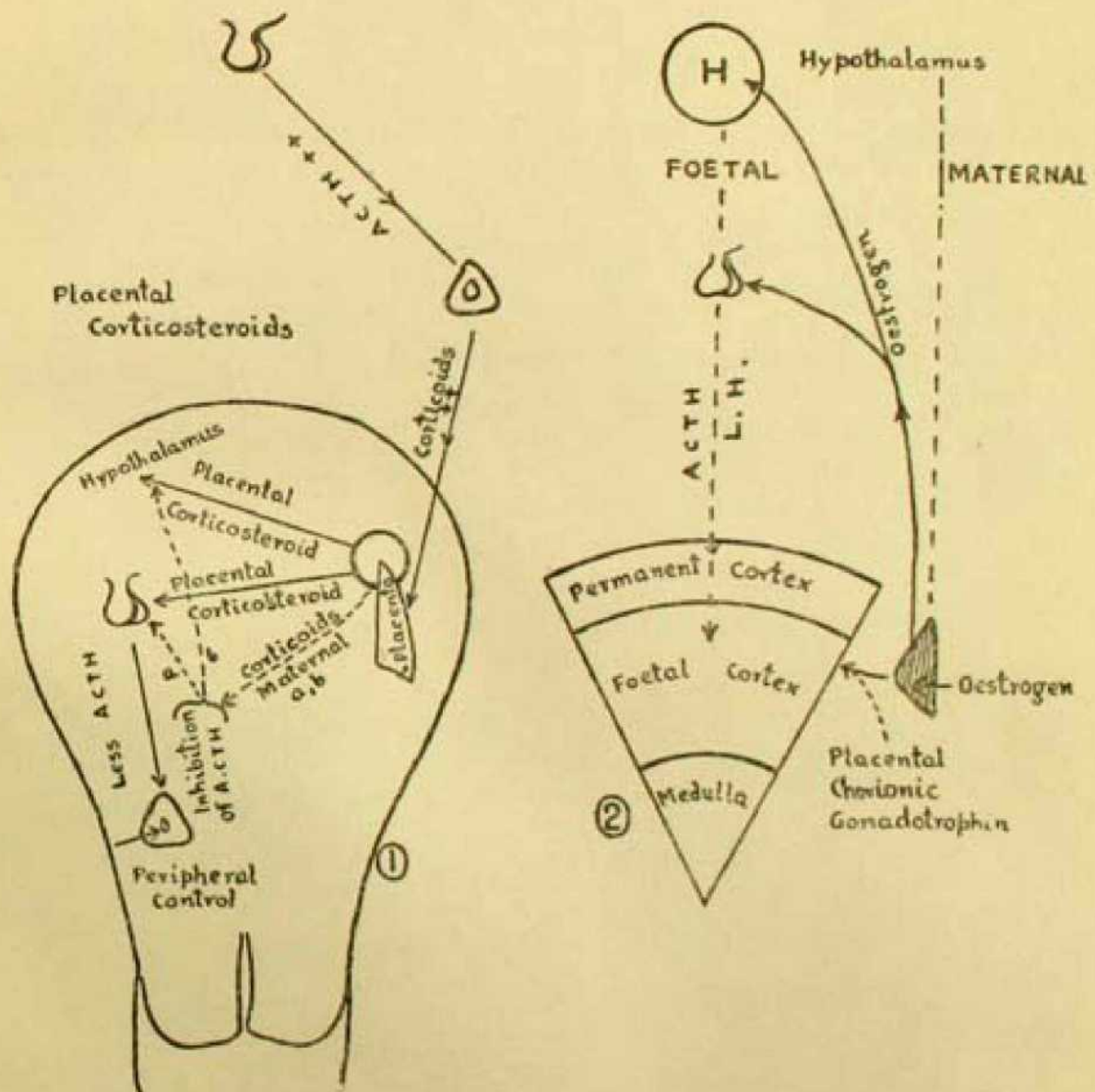


Fig.—8



## CONTROL OF THE ADRENAL CORTEX IN THE ANENCEPHALIC FOETUS

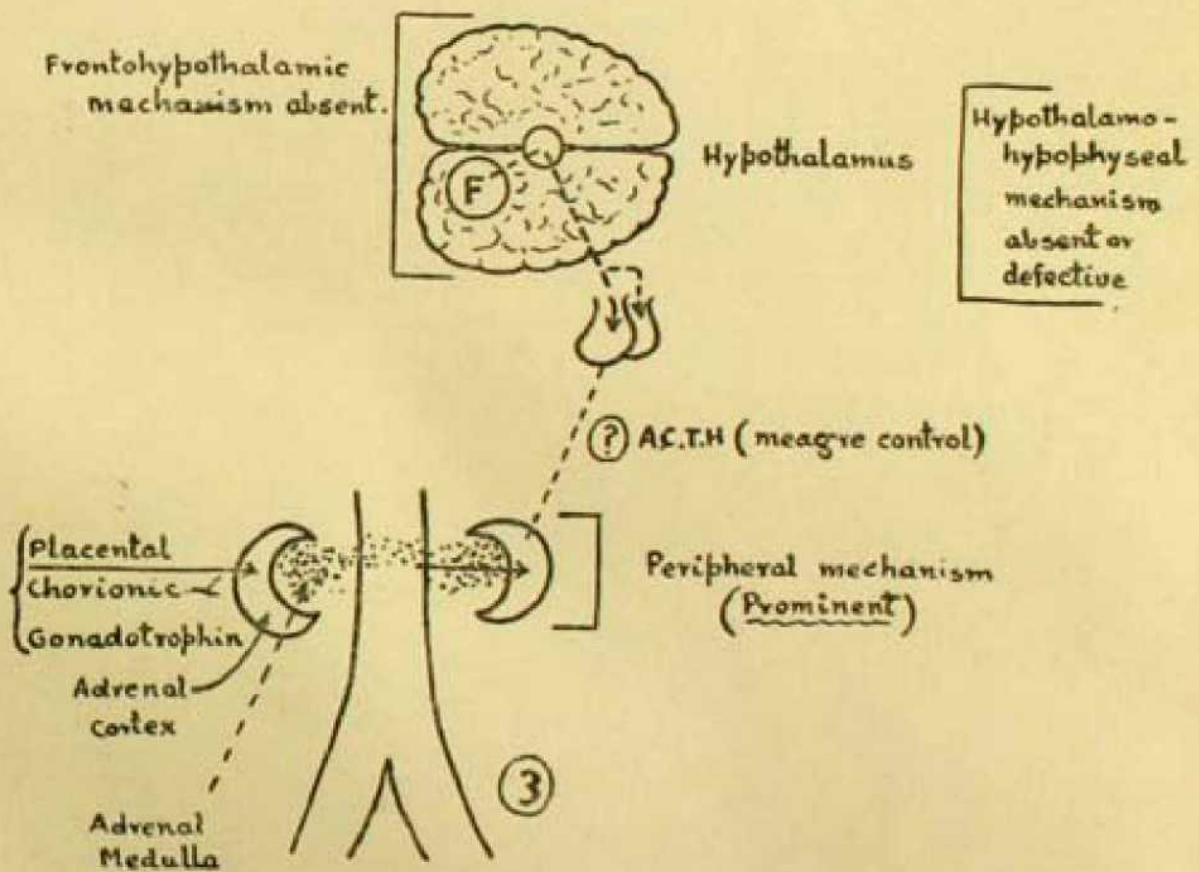


Fig.—9



## CHAPTER—5

### BRAIN MECHANISMS RESPONSIBLE FOR ACTH RELEASE IN THE FISH (1969)

The brain mechanisms responsible for ACTH release in *Ophicephalus punctatus* are described. The study includes both stimulation and lesion experiments in the fish. Stimulation of the olfactory tract, retina, spinal cord, optic tectum, cerebellum and ventral telencephalon is manifested with a rise of plasma 17-OHCS content. The dorsal telencephalon (primordium hippocampi, primordial general cortex, pars striatalis and primordium piriforme) has a checking influence over the pituitary-adrenal-axis. The ventral telencephalon including the primordium amygdalae and the nucleus preopticus has got a stimulatory control over the same axis. Positive response was obtained from the nucleus septalis medialis. The habenula is a modulating centre for the hypothalamus through afferent and efferent paths. Degranulation response of the preoptic cells has been noted after stimulation of the different parts of the brain and spinal cord. The neurosecretory material has both stimulatory and inhibitory control over the pituitary epsilon cells which may secrete ACTH. There was atrophy of gonads in animals with near-total loss of forebrain. Regeneration and reorganisation of the preoptic and lateral tuberal cells occur from ependymal cell layer. Changes in the interrenal cells have been studied in both stimulation and lesion experiments.

#### INTRODUCTION

Achievement of a stable internal environment by the different defence reactions of the body has been called by Cannon (1915) as homeostasis. The sympatho-adrenal system is important for the emergency reactions and homeostasis (Cannon, 1932). Selye (1936) proved the importance of the pituitary-adreno-cortical system in such situations.

Role of afferent nerves in the mediation of the stress response has been studied by Gordon (1950), Hume (1952), Wilson *et al.* (1956), Roy (1960 and 1968), Egdahl (1959) and others.

Importance of the hypothalamus in ACTH secretion has been studied by deGroot and Harris (1950), Hume (1952), Porter (1953 and 1954), Brodich (1963) and others. Brodich (1963) concluded that the entire ventral hypothalamus starting from the optic chiasma to the mamillary bodies was involved in the control of ACTH secretion. The diffuse hypothalamic network regulates ACTH secretion.

Afferents from the oldest parts of the cortex (hippocampus, piriform lobe, cingular gyrus, anterior insula and posterior orbitofrontal region) and of the striatum (amygdala and globus pallidus) reach the hypothalamus (Gloor, 1956). The medial forebrain bundle of the hypothalamus receives fibres from the limbic system by various routes. These extrahypothalamic brain areas control the ACTH release. Hippocampal stimulation depresses adrenocortical activity and abolishes the corticotrophic response to stress in different animals (Porter, 1954; Mason, 1958; Endröczy, *et al.*, 1959; Lissák and Endröczy, 1960; Endröczy and Lissák, 1962). Lesion of the hippocampus leads to a rise of the resting plasma corticosteroid level (Endröczy



*et al.*, 1954 ; Knigge, 1961 ; Roy, 1967). Roy (1967) stated that inhibitory influences over the pituitary-adrenocortical secretion are exerted by the hippocampus, the septum, and the cingulate area. Balancing action is exerted by the cingulate area between the hippocampus and the hypothalamus regarding the pituitary-adrenocortical activity. Dogs with amygdaloid lesion show increased 17-OHCS output in response to burn trauma. He further stated that adaptation activity also occurs at the brain level through different stimulatory and depressive areas of the brain and their afferent and efferent fibre systems. The net result is a steady state of the body in adverse situations, what Claude Bernard has said long time back. Thus apart from the endocrinal adaptation, the central neural integrations are also to be considered.

The above-mentioned review is in relation to mammalian neuro-endocrinology. Brain mechanisms responsible for ACTH release in the fish have not been well explored. Roy (1964) studied forebrain lesions and changes in anterior interrenal cells. There was atrophy of the anterior interrenal cells in *Ophicephalus punctatus* after bilateral ablations of the forebrains.

Peter and Gorbman (1968) observed significant depletion of paraldehyde fuchsinophil granulation of neurones of the preoptic nucleus of the goldfish after electrical stimulation of the olfactory tract, spinal cord and tenth cranial nerve. Retinal stimulation affected the neurones of the pars parvocellularis of the preoptic nucleus. In this way they elaborated some afferent pathways to the preoptic nucleus of the goldfish.

The present study has been undertaken with a view to elaborate the findings of 1964 in relation to the brain mechanisms responsible for ACTH release in the fish. The study includes both stimulation and lesion experiments in *O. punctatus*.

## MATERIAL AND METHODS

*O. punctatus* of four to six inches in length and of both sexes have been used in this experiment. The animals were anaesthetised with 0.01% tricaine methanesulphonate and intraperitoneal injection of flaxedil at a dose of 4 mg/kg for proper immobilization of the fish in stimulation experimental series only. A special holder suspended the fish and fresh water streaming irrigated the gills. For eliciting the "visual grasp reflex" by stimulation of the optic tectum some animals were not injected with flaxedil.

Exposures and electrical stimulation of the olfactory tract, retina, spinal cord and tenth cranial nerve were done after the method of Peter and Gorbman (1968). The frequency of stimulation was 10-15 cps, the duration of the rectangular pulse was 3 msec, the voltage used was from 3 to 5 V. The total time of stimulation varied from 1 to 5 minutes. The indifferent electrode was fixed to the upper jaw during the use of monopolar electrodes.

The control group for the olfactory tract stimulation comprised animals in which the right olfactory nerve was exposed after drilling the skull and nibbling the rest for enlargement of the opening and putting the nerve on bipolar silver electrodes. The nerve was not stimulated and



kept on the electrodes only for the period which was utilised for the stimulation group. The area was covered with mineral oil.

For retinal stimulation, the monopolar silver electrode was kept in contact with the retina at the optic nerve region after removal of structures in front of the retina. In the sham control group, no stimulation was applied.

The sham control for the spinal cord stimulation group comprised the exposure of the spinal cord in front of the dorsal fin and putting the cord on the bipolar silver electrodes. No stimulation was applied for the sham control group.

The control for the tenth cranial nerve comprised the exposure of the nerve very near the medulla and placing it over the electrode without any stimulation.

Electrical stimulation of forebrain was carried out with electrodes of 0.3 mm in diameter with insulation except at the tip. These were bilaterally implanted either superficially or at a depth towards the midline. In the control groups no stimulation was applied. Similarly the optic tectum and the cerebellum were also stimulated.

The lesion experiments comprised :

- (1) surface lesion of the forebrain surgically,
- (2) depth lesion towards the midline by electrocautery, and
- (3) near total ablation of the forebrains surgically.

The animals in the stimulation group were sacrificed after  $\frac{1}{2}$  hour of stimulation and those in the lesion groups at periods specified in the tables. The animals were sacrificed by decapitation. The blood was collected in heparinized tubes. They were centrifuged and the plasma was collected for 17-OHCS estimation as stated by Roy (1964) utilising paper chromatography and Silber-Porter method (1954). The brains and the pituitaries were stained with Gomori's CAHP stain and paraldehyde fuchsin stain. The pituitaries were stained after Cleveland-Wolfe, and PAS-orange G. Adrenals were stained with haematoxylin-eosin, and trichrome stains and PAS. For study of the cellular areas of the brain and fibre systems, serial transverse and sagittal sections were stained after the methods of Klüver and Barrera and Romanes.

## RESULTS

### *Degeneration and regeneration after forebrain ablation :*

After forebrain ablation, evidences of degeneration were noted in tractus strio-tectalis, tractus strio-thalamicus and hypothalamicus, tractus olfacto-hypothalamicus medialis and lateralis, lateral forebrain bundle, tractus olfactohabenularis medialis and lateralis, and tractus preoptico habenularis medialis and lateralis. Degeneration of efferent fibres in fasciculus retroflexus of Meynert was also observed. Some transneuronal degeneration was noted. Regenerative features in the forebrain occurred in the fourth week after surgery.



### Biochemical findings :

Roy (1964) showed that hydrocortisone is the main corticosteroid in *O. punctatus* and production rate of hydrocortisone per gm of adrenal tissue per hour varies according to stress, ACTH, histamine, pitressin and protopituitrin. In the present study, plasma 17-OHCS content of 10 normal fish has been observed to be 12.5 mcg/100 ml (ranging from 18 to 10).

Table I shows the percentage rise of plasma 17-OHCS content (mcg/100 ml) in different controls in relation to the normals in stimulation experiments. In all the controls for the individual groups of experiments, the values are significantly high. This indicates the stimulating effect of anesthesia and surgery during proper exposures for stimulation experiments. Maximum stimulation has been observed in the control group for stimulation of the spinal cord. This shows that fish pituitary-adrenal-axis responds to stress as it does in mammals.

TABLE I

Percentage rise of plasma 17-OHCS (mcg/100 ml) content in different controls in relation to the normals (10) in stimulation experiments.

Olfactory tract stimulation (10)	..	..	..	..	78.4%
Stimulation of Retina (10)	..	..	..	..	95.2%
Stimulation of spinal cord (10)	..	..	..	..	140.8%
Stimulation of optic tectum (10)	..	..	..	..	101.6%
Stimulation of cerebellum (10)	..	..	..	..	128.8%
Stimulation of dorsal telencephalon (10)	..	..	..	..	126.4%
Stimulation of ventral telencephalon (10)	..	..	..	..	113.6%
Stimulation of tenth cranial nerve (10)	..	..	..	..	60.8%

(Number in parenthesis indicates number of animals)

Table II shows percentage rise or fall of plasma 17-OHCS content in *O. punctatus* in stimulation experiments in relation to appropriate controls. During stimulation of olfactory tract, retina, spinal cord, optic tectum, cerebellum, and ventral telencephalon (Figs. 1-6) there is rise of plasma 17-OHCS content. Stimulation of 10th cranial nerve did not show significant rise (16.9%). Stimulation of dorsal telencephalon manifested with a fall of plasma 17-OHCS content and stimulation of the ventral telencephalon manifested with a rise.

Table III shows percentage rise or fall of plasma 17-OHCS content in different controls in relation to the normals in forebrain-lesioned animals. The plasma 17-OHCS values indicate that exposure of the brain is a stimulus for the same at 24 hours. The values reach normalcy towards 7 days, 21 days and 1 month gradually.

Table IV shows the results in animals after dorsal forebrain ablation (Fig. 7). There is rise of plasma 17-OHCS content at 24 hours and 7 days.



The values (percentage rise) at 21 days are significantly lower than at 7 days, and at 1 month the difference with the controls is very meagre.

Table V shows the result after ventral forebrain lesions. In studies at different periods there is fall in plasma 17-OHCS content. However, at one month the percentage of fall is lesser than that observed on previous occasions (Fig. 8).

In table VI the results after near-total forebrain ablation have been presented. In studies at 24 hours, 21 days and at 1 month, there is fall of plasma 17-OHCS content; the maximum fall has been noted at one month (Fig. 9).

TABLE II

Percentage rise or fall of plasma 17-OHCS (mcg/100 ml) content in *stimulation experiments* in relation to *appropriate controls*.

	Rise	Fall
Olfactory tract stimulation (10)	81.1%	
Stimulation of retina (10)	90.9%	
Stimulation of spinal cord (8)	71.7%	
Stimulation of optic tectum (10)	73.4%	
Stimulation of cerebellum (10)	42.6%	
Stimulation of dorsal telencephalon (10)		53%
Stimulation of ventral telencephalon (10)	96.2%	
Stimulation of tenth cranial nerve (7)	16.9%	

TABLE III

Percentage rise or fall of plasma 17-OHCS (mcg/100 ml) content in different controls in relation to the normals (10) in forebrain lesioned animals.

Controls at	Rise	Fall
24 hours (10)	88%	
7 days (10)		14.4%
21 days (10)	No difference	
1 month (10)		1.6%

TABLE IV

Percentage rise of plasma 17-OHCS (mcg/100 ml) content after *dorsal forebrain ablation* in relation to the controls at different periods.

	Rise
24 hours (10)	106.8%
7 days (10)	183.1%
21 days (10)	48.8%
1 month (10)	13.0%

TABLE V

Percentage fall of plasma 17-OHCS (mcg/100 ml) content after *ventral forebrain lesion* in relation to the controls at different periods.

	Fall
24 hours (10)	46.3%
7 days (9)	21.4%
21 days (8)	25.6%
1 month (10)	14.6%



TABLE VI

Percentage fall of plasma 17-OHCS (mcg/100 ml) content after *near-total forebrain ablation* in relation to the controls at different periods.

	Fall
24 hours (10)	45.9%
21 days (10)	34.4%
1 month (10)	56.9%

(Number in parenthesis indicates number of animals)

### NEUROSECRETORY CELLS

The neurosecretory neurons of the preoptic nucleus are filled with paraldehyde fuchsin positive granules normally (Fig. 10). In the individual control groups for the stimulation experiments, there is loss of neurosecretory material from the neurons indicating the stress response to anesthesia and surgery. When electrical stimulation was applied to the different parts of the brain and spinal cord as stated in table II, there was further degranulation response. One very significant feature has been noted when the dorsal telencephalon was stimulated. This manifested with similar degranulation response in spite of the fact that in this series there was a fall in plasma 17-OHCS content. In the control group for the lesion experiments, neurons of the preoptic nucleus show initial degranulation response followed by normalcy at 21 days or at one month (Fig. 11 and 12).

The animals with dorsal forebrain ablation showed degranulation response in the preoptic cells (Fig. 13), but in the third and fourth week the picture was more or less normal. This degranulation response may be due to injury stimulus.

Shock response was noted in the preoptic cells with ventral forebrain lesions (Fig. 14).

In some animals, after stimulation experiments the vessels at the base of the brain showed NSM (colloid).

In near-total forebrain lesioned animals, there was injury response of the tuberal cells with ultimate atrophy of the gonads.

In animals with incomplete forebrain lesions, there is regeneration of the preoptic and lateral tuberal cells. The ependymal cells help in this matter. There were sub-ependymal cells with round nuclei and prominent nucleoli at the end of a month (Fig. 15); but their subsequent development, specially with reference to the atrophied target glands, are required to have a composite view.



## PITUITARY

Oliverreau and Ball (1964) and Ball and Oliverreau (1966) described the epsilon cells of the pars distalis in the teleosts to be the source of ACTH. In the present investigation, these cells have been specially sought for and the changes have been noted in *O. punctatus* in different experimental conditions. These cells occur in the rostral part of the pars distalis. They cannot be stained with PAS and aldehyde fuchsin; but they contain fine erythrosinophilic granules and thus they sometimes escape attention and are taken to be as of chromophobic series. The cells, sometimes oval or columnar in type, are abutted against the neurohypophysis. The epsilon cells of Oliverreau (1964) manifest either normal (Fig. 16) or stimulated picture. The atrophic picture was found in near-total forebrain lesion. The individual experimental groups will not be presented here. During stimulation or stress manifested with rise in plasma 17-OHCS content, the layer of epsilon cells increased in thickness with nuclear and nucleolar prominence. The nuclear membrane was thick. There was degranulation response and vacuolation. At times only the nucleus remained and the cytoplasm was completely replaced by vacuole (Fig. 17). Mitotic figures were scanty. In near-total forebrain ablation, the epsilon cells showed atrophic or involutionary picture. The thickness of the epsilon cells diminished without any prominence of the nucleus and nucleolus. The nuclear membrane was also thin. The standard of erythrosinophilic granules was in between the normal and hypersecreted cells or sometimes the cytoplasm became compact and homogeneous. No mitotic figures were noted.

## INTERRENAL

The interrenal cell in normal *O. punctatus* is short, columnar in appearance with fine granular cytoplasm. The nucleus is round and there are chromatins and a nucleolus (Fig. 18). There is no mitotic activity. In the stimulation experiments and in experiments where plasma 17-OHCS content was found to be high, the interrenal cells showed hypertrophic picture (Fig. 19) with follicular formation. The cells increased in size with increase of layers. The diameter of the nucleus also increased with mitotic figures in some. The nucleolus was prominent. In dorsal forebrain-lesioned animals, within 7 days there was also vacuolar changes in the cytoplasm (Fig. 20). Subsequently at one month, there was normal finding. In animals with near-total forebrain ablation, the interrenal cells showed atrophic picture and this change was marked at 1 month. The cells were small with dark cytoplasm and the small nuclei were dense (Fig. 21).

## VISUAL GRASP REFLEX

On stimulation of the optic tectum of *O. punctatus*, this reflex action as described by Akert (1949) in *Salmo irideus* could be elicited. Tecto-spinal, tecto-bulbar tracts and fibres to the third nerve nucleus are essential for the mediation of this reflex action. In a cranio-caudal sequence, the



muscles involve the movements of eyes, trunk, fins and tail. Movements of the eyes were contraversive, ipsiversive, upwards and others, depending on the different areas of optic tectum, stimulated. The sum total motor effects also depended on the stimulus parameters. This experimental condition mimics how the eye and body movements are oriented when there is food in front/above/below/sides of the animal.

## DISCUSSION

The brain controls the pituitary function through the pituitary stalk. Definite pituitary portal vessels are not found in the fish, but the plexus of vessels between the neuro-and the adenohypophysis may act as portal vessels. Retardation of oviposition was noted by Vivien (1941) in two hypophysectomized female *Gobius* with active pituitary grafts in the anterior chamber of the eye. Roy (1964) noted that in *O. punctatus* with successful autografting of the pituitary in the anterior chamber of the eye, there was atrophy of the gonads, but there was not marked atrophy of the interrenal cells. The autografted anterior pituitary can maintain the interrenal cells. Further, it was observed that hypothalamic lesion manifested with atrophy of the gonads within a month after the operation. There was atrophy of the interrenal cells after bilateral ablation of the forebrains. In incomplete lesions, hypertrophy of the cells was noted. Ball *et al.* (1965) concluded that the ectopic pituitary transplant in *P. formosa* secretes TSH at a higher rate than normal, ACTH at a subnormal rate and growth hormone only in very small amounts. The transplant secretes prolactin-like hormone but does not secrete gonadotrophin. In the grafts, active prolactin cells and TSH cells were plenty and active ACTH cells were present. Few or no active GH cells could be found. The gonadotrophic zone of the graft was occupied by inactive chromophobe cells. Noble (1939) noted that complete removal of the forebrain of the fish had a detrimental effect upon the pituitary with the results that the gonads degenerated. Fish with small rudiment of the forebrain could be brought to spawning by pituitary replacement therapy.

From the stimulation experiments in the present investigation, it has been found that the olfactory tract, retina, spinal cord, optic tectum, cerebellum and ventral telencephalon have stimulatory control over the pituitary-adrenal-axis in *O. punctatus*. The influence of the dorsal telencephalon is inhibitory in nature over the said axis. This has been further proved in animals with dorsal forebrain ablation. However, at one month this inhibitory nature is vanished in the experimental group because of the regeneration of the destroyed areas.

In animals with ventral forebrain lesions, there is fall in plasma 17-OHCS content. Stimulation of the ventral telencephalon led to a rise of plasma 17-OHCS content. Thus, the ventral forebrain has got a stimulatory control over the pituitary-adrenal-axis. In the telencephalon of *O. punctatus* there are, therefore, both stimulatory and depressive areas for the control of ACTH release.



In animals with near-total forebrain ablation, there is maximum fall of 17-OHCS content at one month. This indicates permanent damage to the ACTH secreting mechanism and studies in subsequent months are essential to note the behaviour of the mechanism. This work is in progress.

Fiedler (1967) states that damage to the preoptic area has important effects. The lesion of preoptico-hypophysial tract disturbs the hypothalamo-hypophysial system. There is degeneration of neurosecretory fibres and in the anterior part of the pituitary, there are aggregates of gliocytes due to degeneration of preoptic fibres ending in this area. Tractus preoptico-tuberis and tubero-hypophysial system were lesioned. These experiments were carried out in forebrain-lesioned fish.

Jasinski *et al.* (1966 and 1967) found depletion of paraldehyde fuchsin stainable granules in the cells of the preoptic nucleus of the goldfish after electrical stimulation of the olfactory tract or after perfusion of saline solutions over the olfactory mucosa.

Peter and Gorbman (1968) noticed depletion of paraldehyde fuchsin stainable granules in the cells of the preoptic nucleus of the goldfish after electrical stimulation of the olfactory tract. Stimulation of the spinal cord, tenth cranial nerve and retina exhibited the same degranulation response of the preoptic cells. The pars parvocellularis showed the change on retinal stimulation.

In the present investigation the same type of degranulation response has been noted in the preoptic cells of *O. punctatus* after the stimulation of different parts of the brain and the spinal cord. The spinal cord carries the afferent impulses which ultimately impinge on the preoptic cells. The stress message is therefore carried by the spinal cord to the brain in the fish in a similar manner as is found in mammals. On stimulation of the dorsal telencephalon, there is lowering of plasma 17-OHCS content with the usual degranulation response. Thus, we can state that stimulation of the dorsal telencephalon leads to a dissociated response in relation to the neurosecretory material and plasma 17-OHCS content. This indirectly means that the neurosecretory material of the preoptic cells has both stimulatory and inhibitory control over the pituitary. There was degranulation response of the preoptic cells in ventral forebrain stimulated group. Lateral tuberal cells were also found to be involved in the degranulation response and in animals with near-total forebrain loss, there was ultimate atrophy of the gonads. Neurosecretory material was found to be depleted in the digitations of the neural lobe in stimulated groups. Degranulation response was also found to be present in the groups of dorsal forebrain ablation and in lesion of the ventral forebrain.

Regeneration of the preoptic cells and the lateral tuberal cells occurs in incomplete forebrain lesioned *O. punctatus* at the end of one month. Further studies in relation to the atrophied target glands are essential to note the capacity of the regenerated cells to control the pituitary effectively. Roy (1965) studied the reorganisation of the pituitary stalk after hypophysectomy up to two months in *O. punctatus*. Many of the preoptic cells were absent. Surviving cells had neurosecretory materials in the form



of granules and bigger droplets were also noted. These cells showed transit of NSM along the axons. The proximal stump was reorganised with accumulation of neurosecretory material. Regeneration of the caudal neurosecretory system has been observed by Roy (1962), Fridberg *et al.* (1966) and others. Similar reorganisation and regeneration occur in the preoptic and lateral tuberal cells from a contribution by the ependymal cell layer through some intermediate stages and manifesting *hydroencephalocriny*. Polenov (1954) noted degeneration and replacement of preoptic neurosecretory cells from the ependymal lining in the carp. Polenov (1956) found redifferentiation of lateral tuberal cells from the ependyma in *Cyprinus carpio* and *Abramis brama*. The work of Srebro (1965) leads to the possibility of such a feature in the hypothalamus of the amphibian *Xenopus*.

The pituitary cell type responsible for ACTH secretion has not yet been finally settled. Rasquin (1951) noted changes in the basophil cells of the meso-adenohypophysis after stress. The acidophils were not affected. Rasquin and Atz (1952) and Chavin (1956) found increased percentage of acidophil cells after repeated cortisone injections. The nuclear size increased and there were deeply-stained acidophil granules. Pickford (1957) stated that the findings of Rasquin "throw grave doubts on the theory that corticotropin originates in the acidophils, since profound changes were observed in the basophils. It seems probable that the study of fishes may eventually shed new light on these problems." Farquhar (1957) discovered a sixth cell type in the pars anterior of the rat by electron microscopy. The cells that line the follicles are angular and the position of the nucleus is eccentric. The colloid increases after cortisone injection and decreases after partial adrenalectomy. Herlant (1965) says that the ACTH secreting cells at first seem to be chromophobes, but with tetrachrome staining technique fine erythrosinophilic granules are noted at one pole of the cell. Many experimental findings demonstrate that these cells secrete ACTH. Quenun and Herlant (1964) observed massive hypertrophy of this cell type after bilateral adrenalectomy in the rat. Olivereau (1964) and Ball and Olivereau (1966) concluded that the epsilon cells of the pars distalis in the teleosts are responsible for ACTH secretion. In the present investigation, marked changes have been noted in the epsilon cells of Olivereau in *O. punctatus*. The layer of epsilon cells increased in thickness with nuclear and nucleolar prominence and the nuclear membrane was thick in animals with increased plasma 17-OHCS level. There was degranulation response and vacuolation. In *O. punctatus* with near-total forebrain ablation, the epsilon cells showed atrophic or involuting picture. However, corticotropin secreting nature of the epsilon cells should be verified by SU 4885 test (Metopirone—an adrenocortical inhibitor) and after injection of ACTH and cortisone in *O. punctatus*. The stress response, as has been stated here, is only one side of the answer.

The cytology of the interrenal cells has been described by Rasquin (1951), Olivereau and Fromentin (1954) and the changes in stress have been well reviewed by Pickford and Atz (1957). In *O. punctatus* with high plasma 17-OHCS content, the interrenal cells showed hypertrophic picture with or without vacuolation. Atrophic changes were noted in animals with near-total forebrain lesion. The cytology of the interrenal cells thus



corroborates with that of the epsilon cells and with the lesion or stimulation experiments in the brain along with the discharge or withholding of the neurosecretory material in the neurosecretory centres.

Visual grasp reflex could be elicited in *O. punctatus* with stimulation of the optic tectum and this corroborates with the findings of Akert (1949). For eliciting this reflex action, the tecto-spinal and tecto-bulbar tracts and the fibres to the 3rd nerve nucleus are required.

The terminology of the different areas of the forebrain, the habenula and the dorsal thalamus and the fibre systems has been adopted after Schnitzlein (1962), Fiedler (1967) and Ariens Kappers *et al.* (1936). Ariens Kappers *et al.* (1936) conclude that "All parts of the dorsal olfactory area receive olfactory fibres from the olfactory bulb, and some fibres of this type pass to the olfacto-somatic area, at least its peripheral part. From both the dorsal olfactory area and the olfacto-somatic area, connections are made with the hypothalamic and thalamic regions, largely through the lateral forebrain bundle. The medial forebrain bundle interconnects pre-commissural and septal regions with the hypothalamic areas of the diencephalon. Fibres, probably descending in character, pass through the lateral forebrain bundle to relate the somatic area with the diencephalic regions."

In the brain of the fish, there are stimulatory and depressive areas which control the pituitary-adrenal-axis. The central neurosecretory system controls the pituitary gland of the fish and the hypothalamus is influenced by different brain areas for a control of the pituitary-adrenal-axis. Afferents through spinal cord, retina, olfactory tract, optic tectum, cerebellum and ventral telencephalon activate this system. Areas of stimulation of the dorsal telencephalon included primordium hippocampi, primordial general cortex, pars striatalis and primordium piriforme. The stimulation had a checking influence over the pituitary-adrenal-axis. This is also corroborated by the lesion experiments where one gets release influence over the same axis. Lesion of the ventral telencephalon damages the medial fibre systems and the preoptic cells. This leads to a depression of the pituitary-adrenocortical function. Thus, in the forebrain there are both stimulatory and depressive areas. The pathways involved in this mechanism have been depicted in Figs. 22-26. The habenula seems to be another modulating centre for the hypothalamus through afferent (tractus hippocampo-habenularis, tractus amygdalo-habenularis, tractus septo-habenularis medialis, tractus preoptico-habenularis, and tecto-habenularis) and efferent (habenulodiencephalic and habenulo-peduncular) paths.

### CONCLUSIONS

(i) After forebrain ablation, degeneration was noted in tractus olfacto-hypothalamicus medialis and lateralis, tractus strio-tectalis, tractus strio-thalamicus and hypothalamicus, lateral forebrain bundle, and tractus olfacto-habenularis medialis and lateralis in *O. punctatus*. There were evidences of degeneration in tractus preoptico-habenularis medialis and lateralis and in efferent fibres of fasciculus retroflexus of Meynert. Trans-neuronal degeneration and some regenerative features were also noted.



## STATISTICAL TABLES

TABLE 1

Groups		Average	Range	No. of Observations	S.D.	D.F.
A.	Normal plasma 17-OHCS content	12.5	10.0-18.0	10	2.677	9
B.	Appropriate controls of plasma 17-OHCS content for stimulation experiments of the brain.					
1.	Olf. t. Stimln. .. ..	22.3	16.8-29.0	10	3.901	
2.	Stimln. of Retina .. ..	24.4	17.6-29.7	10	4.124	9
3.	Stimln. of Sp. Cord .. ..	30.1	22.5-36.9	10	4.204	9
4.	Stimln. of Optic tectum .. ..	25.2	17.9-32.1	10	4.450	9
5.	Stimln. of Cerebellum .. ..	28.6	21.8-35.7	10	4.250	9
6.	Stimln. of Dor. telen. .. ..	28.3	22.6-36.5	10	4.062	9
7.	Stimln. of Ven. telen. .. ..	26.7	21.9-32.2	10	3.452	9
8.	Stimln. of 10th Cr. Nerve .. ..	20.1	15.4-26.0	10	3.650	9
C.	Plasma 17-OHCS content after stimulation of :					
1.	Olf. t. Stimln. .. ..	40.4	32.2-49.6	10	5.503	9
2.	Stimln. of Retina .. ..	46.6	36.6-54.8	10	6.726	9
3.	Stimln. of Sp. Cord .. ..	51.7	43.6-60.5	8	6.741	7
4.	Stimln. of Optic tectum .. ..	43.7	34.1-51.2	10	6.728	9
5.	Stimln. of Cerebellum .. ..	40.8	33.2-50.6	10	5.451	9
6.	Stimln. of Dor. telen. .. ..	13.3	9.5-17.0	10	2.370	9
7.	Stimln. of Ven. telen. .. ..	52.4	45.0-61.8	10	5.914	9
8.	Stimln. of 10th Cr. Nerve .. ..	23.5	20.6-28.9	7	2.981	6
D.	Plasma 17-OHCS content in Controls for forebrain lesioned animals at different periods.					
1.	24 hours .. ..	23.5	17.8-28.0	10	5.860	9
2.	7 days .. ..	10.7	7.5-13.8	10	1.934	9
3.	21 days .. ..	12.5	8.9-17.2	10	2.562	9
4.	1 month .. ..	12.3	9.0-16.8	10	2.329	9
E.	Plasma 17-OHCS content after dorsal forebrain ablation at different periods.					
1.	24 hours .. ..	48.6	43.7-53.8	10	3.072	9
2.	7 days .. ..	30.3	20.5-39.4	10	7.261	9
3.	21 days .. ..	18.6	11.4-25.6	10	5.546	9
4.	1 month .. ..	13.9	8.2-22.1	10	4.939	9
F.	Plasma 17-OHCS content after Ventral forebrain ablation at different periods.					
1.	24 hours .. ..	12.6	8.6-16.0	10	2.465	9
2.	7 days .. ..	8.4	6.4-12.0	9	1.802	8
3.	21 days .. ..	9.3	6.7-13.1	8	2.401	7
4.	1 month .. ..	10.5	7.8-13.2	10	1.932	9
G.	Plasma 17-OHCS content after near total forebrain ablation.					
1.	24 hours .. ..	12.7	5.3-19.9	10	5.074	9
2.	21 days .. ..	8.2	4.9-12.5	10	2.790	9
3.	1 month .. ..	5.3	2.5- 9.6	10	2.176	9



Statistical table showing D.F. and values of 't' and their significance for different types of intergroup paired comparisons.

TABLE II

		D.F.	't'
1.	Normal Vs. Control for Olf. St.	18	- 6.550***
2.	Normal Vs. Control for St. of Retina.	18	- 7.657***
3.	Normal Vs. Control for St. of Sp. Cord.	18	- 11.132***
4.	Normal Vs. Control for St. of Op. Tect.	18	- 7.734***
5.	Normal Vs. Control for St. of Cerebellum.	18	- 10.131***
6.	Normal Vs. Control for St. of Dor. telen.	18	- 10.512***
7.	Normal Vs. Control for St. of Vent. telen.	18	- 10.267***
8.	Normal Vs. Control for St. of 10th. Cr. N.	18	- 5.314***

TABLE III

		D.F.	't'
1.	Control for Olf. St.	18	- 8.489***
2.	Control for St. of Retina	18	- 8.901***
3.	Control for St. of Sp. C.	16	- 8.320**
4.	Control for St. of Op. T.	18	- 7.212***
5.	Control for St. of Cereb.	18	- 5.575***
6.	Control for St. of D. tel.	18	- 10.094***
7.	Control for St. of V. tel.	18	- 11.896***
8.	Control for St. of 10th C.N.	15	- 2.032***



TABLE IV

1. Normal Vs. Controls for forebrain lesion 24 hours
2. Normal Vs. Controls for forebrain lesion 7 days
3. Normal Vs. Controls for forebrain lesion 21 days
4. Normal Vs. Controls for forebrain lesion 1 month

18  
18  
18  
18

-5.45\*\*\*  
1.724  
Nil  
0.175

TABLE V

1. Control for forebrain lesion at 24 hours Vs. Dor. forebrain lesion at 24 hours
2. Control for forebrain lesion at 7 days Vs. Dor. forebrain lesion at 7 days
3. Control for forebrain lesion at 21 days Vs. Dor. forebrain lesion at 21 days
4. Control for forebrain lesion at 1 month Vs. Dor. forebrain lesion at 1 month

D.F.

18  
18  
18  
18

$t'$

-12.003\*\*\*  
-8.238\*\*\*  
-3.154\*\*  
-0.919

TABLE VI

1. Control for forebrain lesion at 24 hours Vs. Vent. forebrain lesion at 24 hours
2. Control for forebrain lesion at 7 days Vs. Vent. forebrain lesion at 7 days
3. Control for forebrain lesion at 21 days Vs. Vent. forebrain lesion at 21 days
4. Control for forebrain lesion at 1 month Vs. Vent. forebrain lesion at 1 month

D.F.

18  
17  
16  
18

$t'$

5.427\*\*\*  
2.671\*  
2.709\*  
1.836

TABLE VII

1. Control for forebrain lesion at 24 hours Vs. Near total forebrain lesion at 24 hours
2. Control for forebrain lesion at 21 days Vs. Near total forebrain lesion at 21 days
3. Control for forebrain lesion at 1 month Vs. Near total forebrain lesion at 1 month

D.F.

18  
18  
18

$t'$

4.404\*\*\*  
3.589\*\*  
6.796\*\*\*

\*—Significant at 5% level.  
\*\*—Significant at 1% level.  
\*\*\*—Significant at 0.1% level or more stringent level.



(ii) Stimulation of olfactory tract, retina, spinal cord, optic tectum, cerebellum and ventral telencephalon led to rise of plasma 17-OHCS content in *O. punctatus*. The influence of dorsal telencephalon is inhibitory in nature over the pituitary-adrenal-axis.

(iii) The ventral forebrain has got a stimulatory control over the pituitary-adrenal-axis.

(iv) In animals with near-total forebrain ablation, maximum fall of plasma 17-OHCS content has been observed at one month.

(v) Degranulation response of the preoptic cells has been noted after stimulation of different parts of the brain and the spinal cord.

(vi) Neurosecretory material of the preoptic cells has both stimulatory and inhibitory control over the pituitary.

(vii) Degranulation response was also found in the lateral tuberal cells and in animals with near-total forebrain loss. Ultimate atrophy of the gonads occurred.

(viii) Regeneration and reorganisation of the preoptic and lateral tuberal cells occur from ependymal cell layer and whether such regenerated cells can control the pituitary activity is to be studied further.

(ix) Epsilon cells in *O. punctatus* may secrete ACTH, as changes have been noted in these cells in different experimental conditions.

(x) The interrenal cells showed hypertrophic or atrophic picture depending on the type of experiment. In near-total forebrain ablation, atrophic picture was noted.

(xi) Visual grasp reflex could be elicited in *O. punctatus*.

(xii) The pathways involved in ACTH release in *O. punctatus* have already been stated. The habenula seems to be another modulating centre for controlling the pituitary ACTH release.

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## Chapter—5

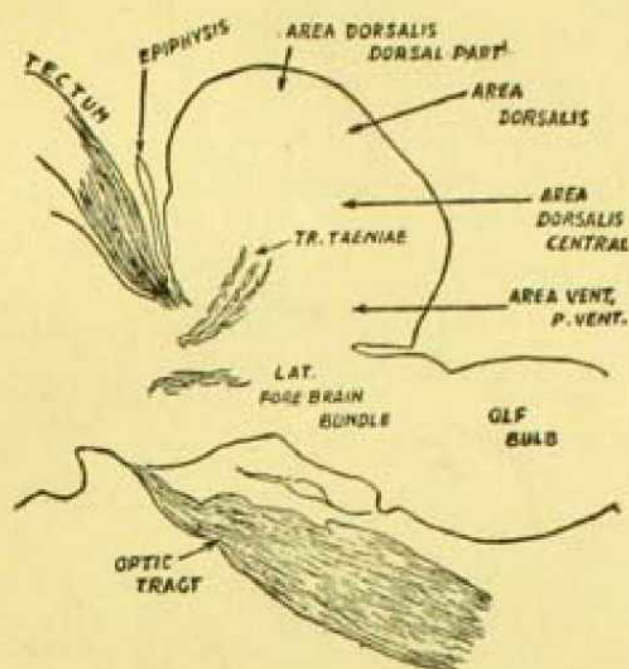


Fig. 2

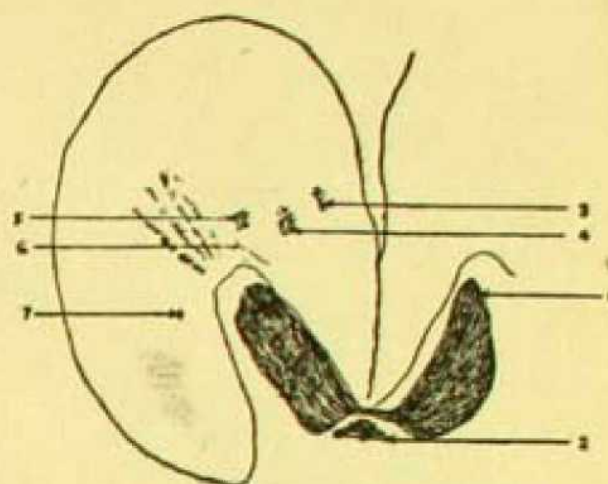


Fig. 4

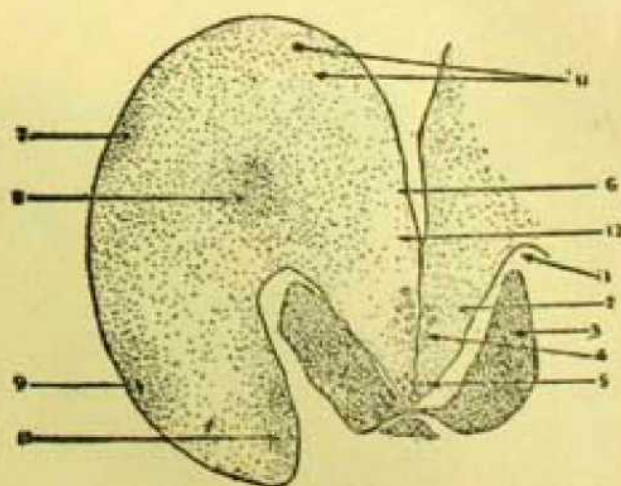


Fig. 3

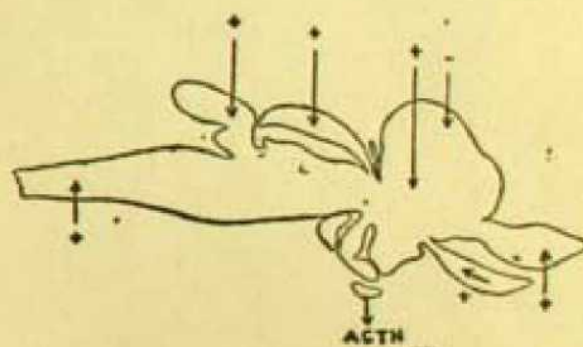


Fig. 5

Fig. 2. Sagittal section of the brain of *O. punctatus* to show the different areas of the telencephalon. Fig. 3. Transverse section of the forebrain of *O. punctatus* to show the nuclear areas :—1. Fissura endorhinalis. 2. Lateral preoptic area. 3. Tractus opticus. 4. Nucleus preopticus magno cellularis. 5. Nucleus preopticus parvocellularis. 6. Nucleus septalis medialis. 7. Primordial general cortex (Dorsal part). 8. Pars striatalis. 9. Primordium piriforme. 10. Primordium amygdalae corticomедial part. 11. Primordium hippocampi. 12. Ventral thalamus. Fig. 4. Transverse section of the forebrain of *O. punctatus* to show the fibre systems :—1. Tractus opticus. 2. Commissura postoptica. 3. Tractus preoptico-habenularis medialis+tr. septo-hab. med. 4. Medial forebrain bundle+tr. hippocampo-habenularis. 5. Lateral forebrain bundle. 6. Tractus preoptico-habenularis lateralis. 7. Tractus amygdalo-habenularis. Fig. 5. Stimulation experiments :— + = indicates rise in ACTH secretion. — = indicates fall in ACTH secretion.



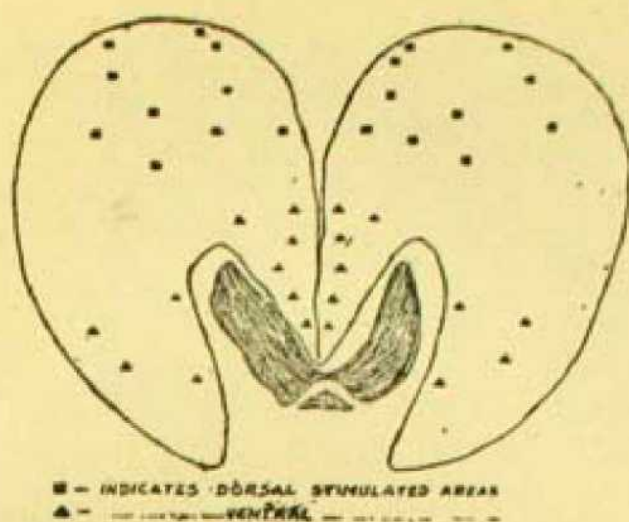


Fig. 6

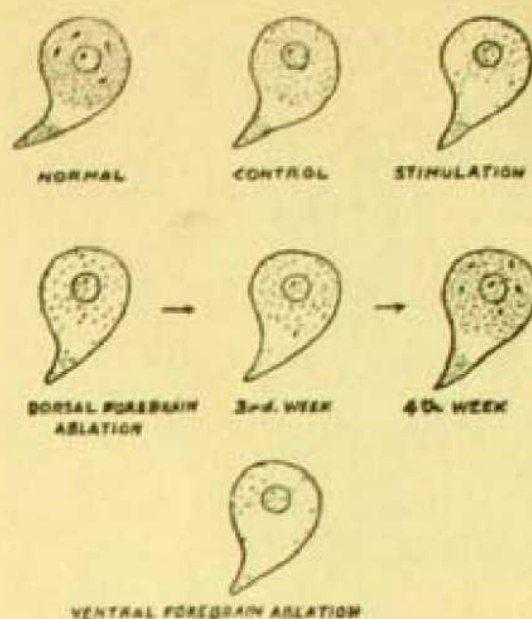


Fig. 12

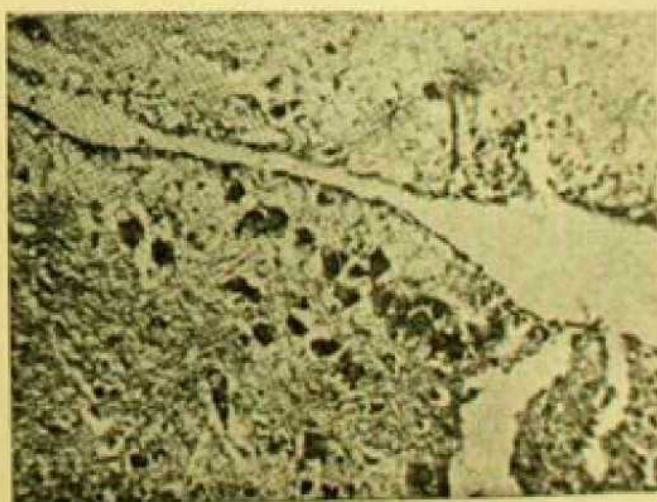
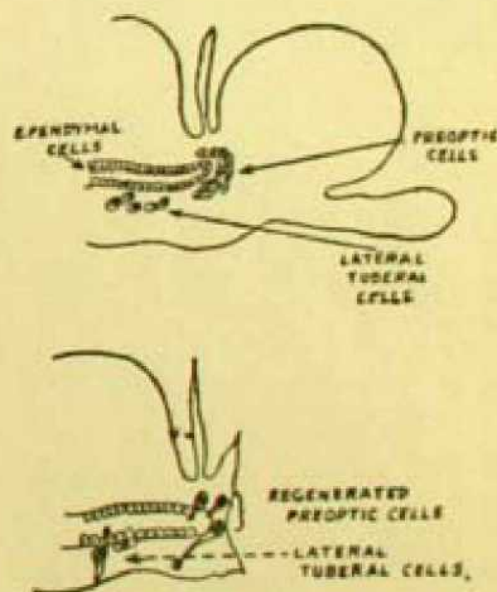


Fig. 11



-Regenerative features in preoptic and lateral tubular cells.

Fig. 15

- Fig. 6. Areas occupied by electrode tips in stimulation experiments (Forebrain).
- Fig. 11. Normal preoptic cells in control group for lesion experiments at the end of one month. Paraldehyde fuchsin stain.  $\times 320$ .
- Fig. 12. Preoptic cells of *O. Punctatus* in different experimental conditions.
- Fig. 15. Regenerative features in preoptic and lateral tubular cells.



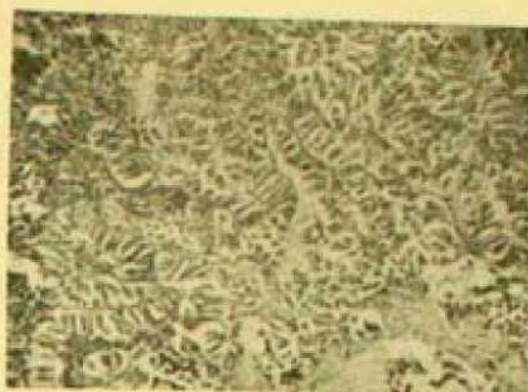


Fig. 19



Fig. 23

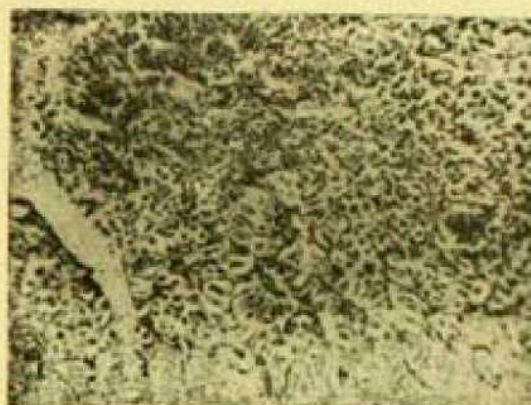


Fig. 20



Fig. 24

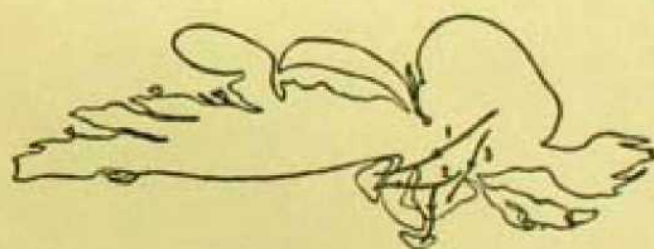


Fig. 25

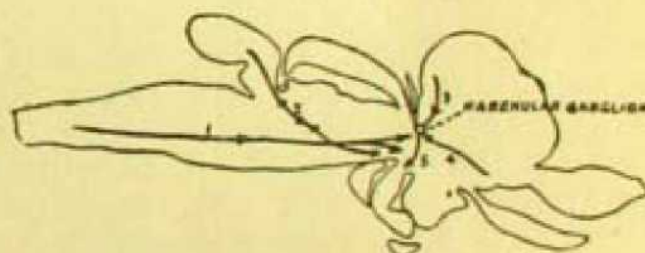


Fig. 26

- Fig. 19. Hypertrophic interrenal cells with follicular formation.  $\times 270$ .
- Fig. 20. Hypertrophic follicular interrenal cells with vacuolation and prominent nucleus with nucleolus. Arrow points to a mitotic figure in the nucleus.  $\times 270$ .
- Fig. 23. Transverse section through nucleus habenularis. Klüver-Barrera stain.  $\times 130$  :—  
 1. Optic tectum. 2. Nucleus rotundus. 3. Optic tract. 4. Commissura transversa. 5. Habenula.  
 6. Stria medullaris. 7. Commissura postoptica dorsalis. 8. Commissura hori-  
 zontalis. 9. Medial forebrain bundle.
- Fig. 24. Sagittal section through the forebrain and thalamus. Klüver-Barrera stain.  $\times 130$ .  
 Shows the fibres of optic tract going to the optic tectum and the nucleus  
 glomerulosus.
- Fig. 25. Neural paths engaged in ACTH release *O. punctatus* (1) :—1. Spinomesencephalic  
 and spinothalamic route. 2. Tractus lobocerebellous and cerebello lobaris.  
 3. Cortico habenular path. 4. Olfacto habenular path. 5. Habenulo peduncular  
 path.
- Fig. 26. Neural paths engaged in ACTH release *O. punctatus* (2) :—1,3. Strio and septotha-  
 lamic and hypothalamic path. 2. Preopicohypothalamic and preopticohypophy-  
 seal path.



## CHAPTER 6

### HISTOLOGICAL AND EXPERIMENTAL OBSERVATIONS ON THE CAUDAL NEUROSECRETORY SYSTEM OF SOME INDIAN FISHES (1962)

#### ABSTRACT

The highly vascular caudal neurosecretory system of some Indian fishes has been described. The neurosecretory cells are developed from the small cells nearby. The praectico-hypophysial system and other spinal cord neurons develop before the caudal neurosecretory system, which has got a cell station, axonal paths for migration of neurosecretory material and the terminal swelling as the storage release centre. The teleosts examined have got the terminal swelling. In *Trygon sphen* there is no terminal swelling but the junctional place between the neurosecretory axon and the blood-vessel functions as the storage release centre. The cells form neurosecretion in cycle and the material may be formed by Nissl granules, basophilic cytoplasm, and the chromatin. The neurosecretory material is discharged in the blood-vessels of the terminal swelling. Both the neurosecretory systems (central and caudal) of *C. mrigala*, *Anabas testudineus* and the *Rita* fish respond to osmotic stimuli. Section of the caudal spinal cord of *C. mrigala* leads to accumulation of neurosecretory material in the proximal stump proving proximodistal migration. There is also neoformation of the terminal swelling with atrophy of the original one. The proximal cells respond to stress. A CRF has been isolated from the caudal neurosecretory system. This system shows much similarity with the hypothalamo-neurohypophysial system.

#### INTRODUCTION

Speidel (1919, 1922) made cytological studies on the caudal spinal cords of skates and other fishes. It was stated that certain neurons may show glandular activity. Weber (1827) described the presence of a warty appendix (knoten) at the end of the spinal cord of the carp and other types of fishes, e.g., the catfish and the burbot. Arsaky (1813), Serres (1826), Zagorsky (1833) and Girgensohn (1846) studied this terminal structure of the spinal cord. Verne (1914) stated the terminal swelling as 'renflement caudal' and Favaro (1926) spoke of it as "ipofisi caudale" (hypophysis caudalis). Kappers *et al.* (1936) state that, apart from glandular cells, septa of connective tissue and blood-vessels of meningeal origin are found in the hypophysis caudalis. This structure is not found in *Murenoids* and *Lophobranchia* and it is best developed in *Culpea*, *Atherina* and *Lophius*. The hypophysis may be divided into two lobes and they are found to curve upwards around the cord.

Dahlgren (1914) studied big cells in the hind part of the spinal cord of skates.

Scharrer (1957) defines the neurosecretory cells 'as a nerve cell which secretes microscopically demonstrable granules via its axon into the blood'. He further says that the neurosecretory cell is the common final pathway for the transmission of messages to organs of internal secretion.



There are four types of neurosecretory systems :

- (1) the crustacean neurosecretory system and the sinus gland as the storage-release centre,
- (2) the intercerebralis-cardicum-allatum complex in insects,
- (3) the hypothalamo-neurohypophysial system in vertebrates, and
- (4) the caudal neurosecretory system in the adult of Japanese species of the eel, *Anguilla japonica* (Enami 1955).

In the system (4) there are anteriorly situated large neurosecretory cells, coarse bundles of neurosecretory pathway and a caudally situated big mass of swollen nerve fibre endings acting as a storage-release centre. This system is well distributed in Pisces (Enami and Imai, 1955, 1956a, b ; Sano, 1958 ; Sano and Knoop, 1959) and the storage depot is termed 'neurohypophysis spinalis' or 'urohypophysis' by Enami (1956). Enami *et al.* (1956) report this to be effective for salt metabolism. Holmgren (1959) found no Gomori-positive material either in the spinal cells or in the urophysis spinalis. The neurosecretory material gave conspicuous 'acidophilic' reaction. The material also gave negative alcian blue and PAS-reactions. Histochemical methods for proteins gave a strong indication that the secretory material is a protein.

Sano (1957) found neurosecretory cells in the anterior grey column of the lumbar part of the spinal cord in several birds.

Speidel (1919) studied few skate embryos of the species *Raia punctata*. The gland cells of internal secretion in the spinal cord of skates were differentiated from the same cells that gave rise to the motor nerve cells of the anterior horn. There was first an increase in the amount and number of chromatin granules. The nucleus became elongated from its original spherical shape. Subsequently the nucleus became lobular due to unequal and localized growth. The chromatin granules also increased. Sano and Kawamoto (1959) described the development of the neurohypophysis spinalis caudalis of *Lebistes reticulatus* Peters, a viviparous bony fish. The first trace of the neurohypophysis spinalis is seen in the form of a dense network of capillaries. At this time the neurohypophysis is fully developed and contains neurosecretory material. The neurohypophysis spinalis is not fully developed before the 20th day after birth and the first trace of neurosecretory material is stainable at about the 14th day. The cells of the neurohypophysis spinalis are probably derived from two rows of specially formed ependymal cells in the floor of the canalis centralis. Gorbman and Ishii (1960) found that in embryos of the shark, *Squalus suckleyi*, differentiation of hypothalamic neurosecretory system was accelerated by treatment with thyroxine, triiodothyronine or triiodothyroacetic acid. Early accumulation of stainable neurosecretory granules is stimulated in the praectico-hypophysial fibre tract, and in the neurohypophysis.

Reviews on osmoregulation have been done by Baldwin (1937), Krogh (1939), Black (1957) and Pickford and Atz (1957).

Enami (1956) studied the changes in the caudal neurosecretory system of the loach (*Misgurnus anguillicaudatus*) in response to osmotic stimuli. He found a state of hypersecretion in the cells after a single intraperitoneal injection.



tion of hypertonic sodium chloride. Repeated injection led to vacuolization and complete loss of secretory activity. After spinal transection in front of the terminal swelling and repeated salt loading there was depletion of neurosecretory material (NSM) in the terminal swelling and then an increased secretory activity in the small cells distal to the site of section. This gave rise to accumulation of NSM in the nerve terminals. When the osmoregulation was greatly impaired, the terminal swelling and the small cells showed depletion of neurosecretory material. There was accumulation of NSM proximal to the level of section and this was elaborated by the more proximally situated cells. This proves increased secretory activity of the cells. In the later stages this accumulation disappeared.

Enami *et al.* (1956) thought of the possibility of occurrence of a sodium regulating hormone in the caudal neurosecretory system of teleosts.

Enami (1959) states : 'In general, many problems remain unsettled in the analysis of the co-ordinating mechanism(s) concerned with the phenomena of Na-exchange, gas-metabolism, and gill morphology. However, if one emphasizes the significance of carbonic anhydrase, the activity of which is known to be related also to the so-called chloride shift, common basic mechanism underlying the three kinds of phenomena might be imagined'.

Aldosterone, an adrenocortical hormone which is present in the fish, undoubtedly helps a lot in osmoregulation.

Histological and experimental investigations regarding the caudal neurosecretory system of the Indian fishes have not as yet been carried out and so these unexplored fields have been taken up.

Corticotrophin releasing factor has been isolated from hypothalamus by many (Hume and Wittenstein, 1950 ; Hellerstein *et al.*, 1952 ; Slusher and Roberts, 1954 ; Guillemin *et al.*, 1957 ; Roy, 1960a ; and others). But no corticotrophin releasing factor has yet been isolated from the caudal neurosecretory system of fishes. This was attempted in the present investigation.

## MATERIAL AND METHODS

### (Development and histology)

The following types of teleost fishes have been examined :

- (1) *Labeo rohita*, (2) *Labeo kalbasu*, (3) *Labeo gonius*, (4) *Cirrhina mrigala*, (5) *Cirrhina reba*, (6) *Catla catla*, (7) *Barbus sarana*, (8) *Barbus ticto*, (9) *Amblypharigodon mola*, (10) *Macrones vittatus*, (11) *Notopterus chitala*, (12) *Wallago attu*, (13) *Lates calcarifer*, (14) *Hilsa ilisha*, (15) *Clarias batrachus*, (16) *Heteropneustes fossilis*, (17) *Anabas testudineus*, (18) *Ophicephalus punctatus*, (19) *Ophicephalus striatus*, (20) *Ophicephalus marulius*, (21) *Mugil tade*, (22) *Mugil parsia*, (23) *Psettus falciformis*, (24) Flying fish—*Exocaetus mento*, (25) *Hemirhamphus*.



One type of chondropterygii has been examined :

(1) *Trygon sephen*.

Fresh specimens were collected from natural sources and local markets of Calcutta and suburbs. Sea fishes were collected from Orissa. Ten adult specimens of each species were studied. For studying the development of the praeoptico-hypophysial system and the caudal neurosecretory system, larvae of *C. catla* and *L. rohita* at different periods were taken. The diencephalon including the pituitary and the caudal spinal cord of the larvae were fixed *in situ* in 10 per cent. formalin or Bouin's fluid. For other purposes the fixatives for the caudal spinal cord were 10 per cent. formalin, Bouin's fluid, Helly's, Levi's and Champy's fluid. The paraffin sections were stained with Gomori's chrome-alum-haematoxylin and phloxin, Mallory's triple stain, toluidine blue, Schiff's reagent for Feulgen reaction, Heidenhain's iron-haematoxylin and eosin method and Van Gieson's method. Photographs were taken from the caudal bodies and the terminal spinal cords. The exact locations of the caudal bodies were marked by pin-head over the particular vertebra and radiographs were taken.

#### OBSERVATIONS

(a) *Development :*

Larvae of *L. rohita* and *C. catla* were studied at different stages of development. At two weeks there is just a starting of the ventral herniation of the caudal part of the spinal cord. The interval between this and the chorda tissue is occupied by a capillary net (Pl.XLV, fig. 1). At this area the meningeal covering is deficient (Pl.XLV, fig. 2). The praeoptico-hypophysial system develops before the caudal neurosecretory system (Pl.LXV, fig. 3). Other spinal cord neurons with Tigroid bodies are developed before the caudal neurosecretory system (Pl.XLV, fig. 4). In these two fishes there is no indication that the ventromedian rows of ependymal cells are directly responsible for the transformation into the caudal neurosecretory cells. Rather, it has been observed that the small cells which are very preponderant may transform into the neurosecretory cells. It may be that the ependymal cells change into the small cells and from these into the neurosecretory cells (Pl. XLV, fig. 2). At two weeks no neurosecretory material is observed in the caudal neurosecretory system. In the third week the neurosecretory material is observed first in the neurosecretory cells and axons and then in the terminal swelling. The terminal swelling appears vacuolated at the stage when the neurosecretory material has not formed in the neurosecretory cells. The praeoptico-hypophysial system is developed in very young larva and Gomori-positive material is observed.

The neurohypophysis is developed from a diverticulum of the diencephalic floor called saccus infundibuli. Wingstrand (1959) stated that 'The oral surface of the saccus infundibuli, or the base of it, together with parts of the post-optic floor, develops into the neurohypophysis. It is characterized by its close connection with the fibres of the neurosecretory



system.' In the development of the caudal neurosecretory system of the two types of fishes studied here, there is no indication of such diverticulum formation from the central canal of the spinal cord. In *Wallago attu* the development of saccus infundibuli-like structure in the dorsal part of the terminal swelling prior to its formation is possible.

Close approximation of the neurohypophysial with the adenohypophysial component is important for the proper control of the adenohypophysis by the hypothalamus. But it has been shown by Etkin (1958) that, if the adenohypophysial anlage is removed in the frog tadpole in stage 17 and transplanted into the tailbud, the proper development of the neurohypophysis and adenohypophysis occurs. This shows that the contact of the two parts is not essential for proper development when the primordia are formed. The pituitary-grafted animals are black because of increased secretion of intermedin and they grow faster than non-grafted animals and become giant sized because of excessive production of growth hormonees. This shows that hypothalamic control is essential for finer adjustment of the release of anterior pituitary hormones. In the caudal neurosecretory system there is no adenohypophysial component and therefore this link is missing ; but a feed-back mechanism may operate here from target glands.

The factors which lead to the herniation of the caudal spinal cord for the development of the terminal swelling are unknown at present. In the spinal cord transection experiment it is seen that there is a neoformation of the terminal swelling-like structure proximal to the level of section. This is due to the accumulation of neurosecretory material proximally after its elaboration from the cells and propagation along the axons. This now acts as a new storehouse. The original terminal swelling atrophies and is vacuolated. At first there is a leash of newly-formed capillaries and surrounding these the neurosecretory material accumulates. These capillaries may have some chemotactic means by which the neurosecretory material comes around them or the neurosecretory material migrates along the axons by axoplasm current. A similar account may explain the starting of the herniation process.

This type of neoformation of the terminal swelling is also met with in the pituitary stalk region after the ablation of the hypophysis and this newly-formed neurohypophysis responds to stress. The newly-formed terminal swelling also responds to stress (Enami, 1956).

(b) *The glomus coccygeum and the terminal swelling of the fish spinal cord :*

As both these structures are situated in the hind part of the body, one may think that the coccygeal medullary vestige is the same as the terminal swelling of the fish spinal cord. The difference is as follows :

(i) *Developmentally*—They are separate. The coccygeal body is an arterio-venous anastomosis and it is developed in connection with the middle sacral artery. It develops as a channelled mass and its polyhedral cells have been thought to be either as highly modified smooth-muscle elements or as post-embryonal angioblasts.



(ii) *Anatomically*—The glomus coccygeum lies ventral to the coccyx in close relation to the arteria sacralis media.

The terminal swelling is situated in the spinal canal in association with the spinal cord.

(iii) *Histologically*—The terminal swelling of the spinal cord of fishes is a storehouse of the neurosecretory substance elaborated by the caudal neurosecretory cells and transported to the storehouse along axons. The neurosecretory substance is accumulated around blood-vessels.

The glomus coccygeum consists of a vascular plexus rather than chromaffin tissue. It is not an end-station for the neurosecretory substance coming from the neurosecretory cells situated in the caudal spinal cord.

(iv) *Functionally*—The caudal neurosecretory system including the terminal swelling has got the following functions :

- (1) It takes part in sodium exchange (Enami, 1959).
- (2) It has got relation to gas metabolism (Enami, 1959).
- (3) It has got action on gill morphology (Enami, 1959).
- (4) It has got action on the pituitary-adrenocortical axis. This has been found in the present investigation.

No such action is ascribable to the presence of glomus coccygeum. Moreover, there is no indication of gradual atrophy of the terminal swelling with increase of age. Atrophy occurs only when it is transected by surgery proximally. Thus it shows that this swelling has got some important functions.

#### (c) *Vascularity of the caudal neurosecretory system :*

The caudal spinal cord and the terminal swelling in the fishes examined are highly vascular. The neurosecretory cells in the caudal spinal cord are surrounded by blood capillaries and blood-vessels are also situated endocellularly. The neurosecretory substance is present in these vessels. Sometimes the endocellular vessel traverses the cell in such a way as to divide it into two halves, one half containing the nucleus and the other half devoid of it (Pl. XLVI, fig. 5). The nucleus comes in contact with the capillary. The vascular contact of the nucleus with the cytoplasm may have some role in the cellular metabolism. Similar peri- and endocellular capillaries have been noted in the hypothalamic neurosecretory cells of these fishes. Because of the high metabolic rate in these cells, they may require more nutritive substances and oxygen and so they are richly vascularized. Moreover, an alteration in the physical or chemical nature of the blood will alter the cellular activity. This type of control is present in the hypothalamic neurosecretory cells and similar control is expected in the neurosecretory cells of the caudal spinal cord of fishes. That the neurosecretory cells in the hypothalamus are supplied by blood-vessels have been mentioned by Collin (1931a, b), Scharrer and Gaupp (1933), Scharrer and Scharrer (1954a, b) and Roussy and Mosinger (1937). Sano and Kawamoto (1960) described endocellular



capillaries in the neurosecretory cells of the caudal spinal cord of *Channa argus* Cantor. Scharrer (1955) discussed about the factors which could determine capillary density. It does not depend so much on the number and size of the cells but on the number of synapses. This accounts for the poor vascularity of the spinal ganglia. Though the cells are densely packed, no synapses are found. Then he mentions that 'if the number of synapses were the determining factor, neuropils, i.e., areas of interwoven nerve fibres and synaptic endings such as the molecular layer of the cerebellum of the vertebrates, the so-called nucleus rotundus of the fish brain, and so forth, should show relatively dense capillary beds.' But differences are there. Poor vascularization has been noted in some neuropils, like the molecular layer of the cerebellum. In the nucleus rotundus of the fish brain there are dense capillary beds. The different neuropils differ according to the number of mitochondria in the synapses. Mitochondria are rich in neuropils with many capillaries. Few mitochondria have been noted in neuropils with poor vascularity. Enzymes are carried in mitochondria and differences in the number of mitochondria in different areas of the brain probably signify differences in metabolism and thus there is variation in vascular density.

The terminal swelling of the caudal spinal cord of the fishes examined is vascular and neurosecretory material has been found in the vessels. This is a type of transport of the neurosecretory material from the storage-release centre. The quantity of the neurosecretory material in the vessels is variable depending on the type of demand. In the ventral and ventrolateral aspects of the caudal spinal cord there is good amount of vascularity. Axonal processes of some of the neurosecretory cells end around these vessels instead of coursing into the storage organ. This is another mode of discharge of the neurosecretory substance.

#### (d) Histological findings :

Separate terminal swelling was present in all the varieties of fishes (except *Trygon sephen*) examined and experimented with as the particular type of storage-release centre whereas in *Trygon sephen* the junctional area between the end of the neurosecretory axon and the blood-vessels serves the same purpose.

The terminal swelling is situated on the ventral aspect of the spinal cord. It rests in a depression of the body of the vertebra and has a very good blood supply. According to Favaro there are six different types of the terminal swelling :

- (a) single ventral swelling,
- (b) unpaired ventrolateral swelling,
- (c) ventral swelling divided into lateral halves,
- (d) paired ventral swelling,
- (e) lateral bulges, and
- (f) dorsally united lateral bulges.

In the present investigation all of the above types have been encountered except type (f). In *C. mrigala* the surface of the terminal swelling seems to be nodular. In *Catla* the swelling is not round but rather fusiform in shape.



In *Wallago attu* the central canal expands at the dorsal part of the terminal swelling just like the infundibular recess of the third ventricle near the posterior pituitary (Pl. XLVI, fig. 6). There is no terminal swelling in *Trygon sephen*.

The exact location of the terminal swelling is shown in Pls. XLVI and XLVII, Figs. 7, 8, 9, 10, by a pin-head. The different types of the terminal swelling is shown (Pls. XLVII, XLVIII, XLIX, L, Figs. 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24).

The terminal swelling is not considered to be a discrete body at present but it represents a part of a well-organized system which is known as the caudal neurosecretory system. The NSM is formed in cells situated more proximally, carried along axons, stored in the terminal swelling around blood-vessels and is discharged into the lumen of the blood-vessels.

#### *Neurosecretory cells :*

The neurosecretory cells in the caudal spinal cord are like the praeoptic nuclei and nucleus lateralis tuberis of fishes. These cells are big and have got polymorphic nuclei. They produce secretory material and are richly supplied with blood. Big cells are situated more proximally and small cells are more distally located near the terminal swelling. Cells with single nucleus and nucleolus are also encountered. In the cytoplasm the neurosecretory material is stained with acid fuchsin part of Mallory and Van Gieson stains. With Gomori's chrome-alum-haematoxylin-phloxin stain the neurosecretory material takes up the phloxine component. With iron-haematoxylin it is blue-black.

#### *Secretion cycle :*

Some cells are seen to be filled with neurosecretory material while others are devoid of it and are vacuolated and still in others scanty amount of neurosecretory material is noted (Pl. LI, Fig. 25). From these different pictures it is understood that a secretion cycle takes place in these neurosecretory cells with elaboration of secretory material.

#### *Nissl substance and neurosecretory material :*

Nissl substance is present in the neurosecretory cells and the observations indicate that the Nissl substance may give rise to the neurosecretory material. Four different types of cells are found. In one, the neurosecretory material is peripherally located amongst the Nissl substance. In the second variety it is perinuclear and in the third one the whole cytoplasm is filled up with neurosecretory material. In the fourth variety the neurosecretory material is found in vacuoles in the cytoplasm. Scharrer *et al.* (1945) studied the Nissl substance in secreting nerve cells. They found inverse relationship between the amount of Nissl substance and that of neurosecretory



material. There are other different ways by which the Nissl substance gives rise to the neurosecretory material. They are as follows :

- (a) Vacuoles are formed in the cell periphery among the Nissl substance. They are filled either with homogeneous substance or with red staining granules.
- (b) The Nissl granules may be transformed into homogeneous acidophil material at the periphery of the cell.

Such types of progressive transformation from the Nissl substance to the neurosecretory material have been observed in the caudal neurosecretory cells of the fishes studied here. This has also been observed by Bargmann (1949) and Hild (1950). This inverse relationship is also noted amongst invertebrates and insects (Gabe, 1951, 1953 ; Arvy and Gabe, 1952a, b). Ito and Oishi (1950) did not find any definite clue that the neurosecretory granules are formed at the cost of Nissl substance. The neurosecretory material and the Nissl substance are Feulgen negative. The nuclear chromatin is Feulgen positive.

#### *The basophilic cytoplasm and the neurosecretory material :*

The basophilic cytoplasm is located about the nuclear surface in the caudal neurosecretory cells. With the increase of neurosecretory material there is disappearance of the basophilic cytoplasm.

#### *Nucleus and the neurosecretory substance :*

The nucleus of the caudal neurosecretory cells studied here is usually spherical. In some nuclei there are indentations or notched appearances and these are lined by basophil cytoplasm. Nucleus may also be of cup or crescent shape. The concave surface is filled in by basophil cytoplasm. Similar features were observed in the nucleus lateralis tuberis of fishes by Palay (1943). Each nucleus has got one nucleolus but multiple nucleoli may also be found. There are chromatins in the nucleus and the findings indicate that the neurosecretory substance may be produced in the nucleus at the expense of chromatins. Nuclei in different states of elaboration of the neurosecretory material are found. Some nuclei appear absolutely empty (Pl. LI, Fig. 26) indicating that the secretion has passed into the cytoplasm. In some the neurosecretory material is noted amidst the chromatins (Pl. LI, Fig. 27).

Multilobed nuclei have also been noted. Finger-like projections start from the nuclei. These are specially found in *Trygon sephen*. In these complicated nuclei, chromatins and multiple nucleoli are found. The chromatins are finely granular. Basophil cytoplasm occupy the spaces between the lobes. The nuclear membrane is distinct on the convex surface but it is indistinct at the finger-like process or in between the multiple lobes. The intranuclear inclusions are in the form of granules or globules. The smaller granules unite to form the globules. The globules are discharged into the cytoplasm through the concave border of the complicated nuclei. Palay



(1943) described three possible ways by which the inclusions are discharged from the nucleus into the cytoplasm :

(1) They may exude out of the nucleus through the delicate or defective membrane which covers the nuclear process and the filamentous interconnections between lobes.

(2) They may be extruded into nuclear invaginations lined with dark staining cytoplasm.

(3) They may be expelled as a cluster through a large defect in the nuclear membrane.

Scharrer and Scharrer (1954a) state that chromatins also give rise to secretory granules in the neurosecretory cells. The secretory granules increase when the chromatins decrease.

*Pathway for transport of neurosecretory material from the neurosecretory cells to the storehouse :*

The neurosecretory cell groups in the caudal spinal cord send thick axons up to the terminal swelling. Some axons stop more proximally. On these axons neurosecretory material is accumulated in beaded fashion. These beaded accumulations have got the same tinctorial characteristics as those of the neurosecretory material in the cells. Thus it seems that the neurosecretory material is transported along these axons into the storage-release centre, i.e., the terminal swelling.

More definite proof about the passage of the neurosecretory material along the axons is noted from the transection experiment of the caudal spinal cord.

*Storage-release centre :*

The terminal swelling acts as the storage-release centre. The neurosecretory material elaborated in the neurosecretory cells is transported along the axons and accumulates at their ends surrounding blood-vessels. Neurosecretory material is found in the lumen of such blood-vessels in the storage-release centre (Pl. LI, Fig. 28). Enami (1955) described such features in the eel.

There is no cellular element in the terminal swelling other than the connective tissue elements. There are some glia-cells and Herring bodies.

In the caudal bodies examined so far, there has not been found even any solitary incidence of the presence of the anterior pituitary-like tissue.

The neurosecretory material takes up reddish colour in Mallory, Van Gieson and Gomori staining. It is Feulgen negative.

Thus it is definite that the terminal swelling is not an isolated entity but it is a part of a system which is known as the caudal neurosecretory system (Enami, 1955).



*Dahlgren's cells in the spinal cord of Trygon sephen :*

Speidel (1919) described the gland cells of internal secretion in the spinal cord of the skates. Fridberg (1959) studied the Dahlgren's cells in rays and teleosts. The cells have got processes which penetrate the glial membrane of the spinal cord into a highly vascularized part of the endomeninx in the ventral fissure of the cord and dorsal to the spinal artery. The processes have got bulbous swellings which are in intimate connection with the vessels. The processes of Dahlgren's cells act as secretory storing and releasing centre like the neurohypophysis spinalis caudalis in teleosts (Sano, 1958). Bern and Hagadorn (1959) described the caudal neurosecretory system in the elasmobranch—*Rhinobatos productus*, the shovelnose guitar fish. They suggest that in elasmobranchs the more primitive homologue of the urohypophysis may be a diffuse area where the secretion comes in contact with capillaries in the ventral surface of the spinal cord. This area is situated where the giant neurosecretory cell bodies are found.

In the hind part of the spinal cord of *Trygon sephen*, Dahlgren's cells have been noted. The cells are very big in size and are situated on both sides of the central canal. The cells may be of elongated or irregular shape. The long axes of the cells are disposed either anteroposteriorly or dorso-ventrally. Processes extend from the cells and abut against a vessel on the ventral side. Gomori-negative neurosecretory material is seen along the processes and at the ends of them, abutting against the wall of the blood-vessels presumably to be discharged into the lumen. Thus the junctional place between the ends of the processes and wall of the blood-vessel acts as a storage-release centre just like the terminal swelling studied previously. This is because of the fact that *Trygon sephen* has got no terminal swelling. The giant cells have got complex nuclei. These are multilobed but mononuclear cells are also met with. The nuclear membrane is distinct on the convex side and indistinct on the concave one. Large chromatin granules are found within the nucleus. Intranuclear neurosecretory material is found in the form of globules (Pl. LII, Fig. 29). These are extruded into the cytoplasm (Pl. LII Fig. 30). This finding speaks for the elaboration of the neurosecretory material by the nucleus. The resting cytoplasm is homogeneous but in an active cell vacuoles of different sizes with or without neurosecretory material are noted. A cell with multiple small vacuoles is found. Another cell with a big vacuole and with or without neurosecretory material within it is noted (Pl. LII, Fig. 30). These when placed side by side mean that the small vacuoles coalesce to form a big vacuole with appearance or release of neurosecretory material. Moreover, the neurosecretory material is noted in the cell cytoplasm outside the vacuoles (Pl. LII, Figs. 30, 31). The neurosecretory material is never found lying free in the cord tissue. Speidel (1919) noted increase in volume of granular material after electrical or pilocarpine stimulation of the spinal cord. No increase in volume of granular material was seen after atropine stimulation. This indicates that the cells are gland cells of internal secretion. The exact function of the cells is unknown. He suggests that experiments are to be done with extracts of granular secretion.



We find that in *Trygon sephen* the neurosecretory material is elaborated in the giant cells, passes along the processes, and is stored in the storage-release centres, to be discharged when necessary. Thus it is a well-organized system having some definite functions for the benefit of the body.

## EXPERIMENTAL

### (A) Osmotic Stimuli and Neurosecretory System

#### (a) Material and methods :

*C. mrigala* of about 10 inches in length and 18 in number has been used in this experiment. These are freshwater teleosts and cannot tolerate higher percentage of salinity well. Maximum percentage of sodium chloride solution used here is 7.5. Other strengths used were 0.9, 1.8, 2.5 and 5 per cent. The period of study was 1 h, 2 h and 4 h. The animals were gradually transferred from lower to higher salinity. Towards the higher salinity the observation period was very short (five to ten min.) and then some of the animals were transferred back to fresh water. Surgical section of the caudal spinal cord in front of the terminal swelling was done in 10 other animals. These were then subjected to the osmotic stimuli. After the experiments were over the diencephalon, the pituitary and the caudal spinal cord including the terminal swelling were fixed in Bouin's fluid and 10 per cent. formalin and paraffin sections were stained with Gomori's chrome-alum-haematoxylin-phloxin stain and trichrome stain of Masson.

*Anabas testudineus* of five inches in length and 15 in number and *Rita* fishes of about eight inches in length have also been used. The *Rita* fish has been collected from the Ganges at Varanasi. These two types of fish have been included here as they can survive out of water. Moreover, they can tolerate more concentrated sodium chloride solutions. The sodium chloride solutions used are of 0.9, 1.8, 2.5, 5, 7.5, 10 and 15 per cent. The *Rita* fish could survive in 15 per cent. solution for 11 h. *Anabas testudineus* could survive at 15 per cent. solution only for six min. when they were transferred from the lowest to the highest concentration through the intermediary ones. In each solution the fish was kept for 10 min. and then transferred to the next higher concentration.

Materials for histological study, fixation of tissues and staining procedures are same as mentioned for *Cirrhina mrigala*.

#### (b) Observations :

*The praeoptico-hypophysial system in C. mrigala.*—The neurosecretory content of the praeoptic cells varies in normal condition. Some cells are completely filled up with NSM and some are empty. The praeoptico-hypophysial tract takes up chrome-alum-haematoxylin stain. Beaded appearance of the fibres is also seen. Presence of Herring bodies is seen in the pars nervosa. The detailed histology of the pituitary of *C. mrigala* has been already described (Roy, 1961, *in press*). The caudal neurosecretory system of *C. mrigala* has been described in this paper.



*The influence of hypertonic saline on the praeoptico-hypophysial system, nucleus tuberis lateralis, and the caudal neurosecretory system of C. mrigala.*

*The praeoptico-hypophysial system.*—There is a loss of NSM in the neuro-hypophysis. The Herring bodies are scanty or absent in some. Loss of neurosecretory material from the praeoptico-hypophysial tract is noted. The praeoptic cells are free from neurosecretory material (hyperfunctioning stage) (Pls. LII, LIII, Figs. 32, 33).

When the fishes were transferred to fresh water, there was reappearance of NSM in the praeoptico-hypophysial system. The appearance of NSM started first in the neurohypophysis, then in the praeoptico-hypophysial tract and lastly in the praeoptic cells.

In the cells of the nucleus tuberis lateralis there was no marked change due to the osmotic stimuli.

*Caudal neurosecretory system.*—There was depletion of NSM from the terminal swelling (the storage-release centre), the axons and the caudal neurosecretory cells. Cells with vacuolated appearance were plenty (Pl. LIII, Fig. 34).

Early accumulation of NSM in the proximal stump of the cord in cord-sectioned animals with its subsequent disappearance after osmotic stimuli proves the axonal migration of the NSM and also that such a lesioned system responds to osmotic stimuli. This fully agrees with the findings of Enami (1956).

When the fishes were transferred to fresh water, there was reappearance of neurosecretory material in the caudal neurosecretory system. The appearance of NSM was identical to that observed in the praeoptico-hypophysial system.

After osmotic stimuli there was depletion of neurosecretory material from the praeoptico-hypophysial system and the caudal neurosecretory system of *Anabas testudineus* and the *Rita* fish (Pls. LIII, LIV, Figs. 35, 36, 37) but there was no change in the cells of the nucleus tuberis lateralis (Pl. LIV, Fig. 38).

(c) *Discussion :*

Arvy and Gabe (1954), Arvy *et al.* (1954), Tuurala (1957) and Fridberg and Olsson (1959) found decrease of neurosecretory substance in the praeoptico-hypophysial system of fishes when they were transferred from fresh water to salt water. In higher vertebrates neurosecretory material contains an anti-diuretic principle (Bargmann, 1954). The above-mentioned observations confirm this in fishes. Arvy and Gabe (1954), Arvy *et al.* (1954), Tuurala (1957) and Rasquin and Stoll (1957) found accumulation of neurosecretory material in the neurosecretory system due to hypotonic condition. But Fridberg and Olsson (1959) did not find it. They think that this may be due to a stress reaction. Rasquin and Stoll (1957) found accumulation of neurosecretory material in some brain nuclei and also in nucleus lateralis tuberis after injections of water or Pitressin. Fridberg and Olsson (1959) found no



connection between either the production of secretion in nucleus lateralis tuberis or in the subcommissural organ and variations in osmotic value. Korn (1960) studied changes in the praectico-hypophysial system of *Mugil* and *Gobius* in hypotonic medium.

Verney (1947) spoke about the large vesicles situated in the neurosecretory cells of the supraoptic nuclei in dogs as osmoreceptors. This occurs only in a few species. The observations of Bargmann *et al.* (1950), Hild and Zetler (1953a) and Jewell (1953) indicate that such a type of vesicle depends on the functional condition of the cell. Euler (1953) injected hypertonic solution of sodium chloride (2 per cent) and glucose (10 per cent) into the carotid artery of cats and found electrical activity in the supraoptic region. Ortmann (1951) found depletion of neurosecretory material after dehydration, thirst, overloading with sodium chloride and heat. Alteration in the nucleus of the hypothalamic neurosecretory cell was noted after thirst by Eichner (1952, 1954) and Macher (1952). Neurosecretory material is depleted first in the posterior lobe. With thirst, depletion of NSM occurs in the rat in 12 to 14 days and in the dog in 7 to 14 days. Ortmann (1951) found that, even when the neurosecretory material was extensively depleted, some unaffected Herring bodies were found in the hypothalamic nuclei and in the posterior lobe for a long time. Eichner (1952), Hillarp (1949), and Macher (1952) found nuclear enlargement. Ortmann (1951, 1960) found nuclear enlargement, eccentric position of the nucleus, enlargement of nucleoli, dissolution of the Nissl material and ultimately degeneration of the cell. These changes were found at the same time when the neurosecretory material was depleted. Ortmann (1951) found increased mitotic activity of the posterior lobe pituicytes in rats. The changes are reversible. Time for reaccumulation of NSM is usually longer. Ortmann (1960) states: 'It should be pointed out that each of these cellular changes is similar to the axon reaction and may represent an increased nervous activity as well as secretory activity of the cell.' When saline drinking water was chronically administered, there was an increase in NSM in all parts of the hypothalamo-hypophysial system and this is interpreted as 'an adaptation with subsequent hyperfunction'. Hild and Zetler (1951, 1952a, b, 1953a, b) and Hild (1956) found a parallelism between the hormone content of the supraoptic and paraventricular nuclei, the supraoptico-hypophysial tract and the neurohypophysis on the one hand and the amount of histologically stainable material present in sections on the other. Neurosecretory substances (carrier substance and hormones) are depleted from the hypothalamo-hypophysial system by dehydration of the experimental animals. On re-establishment of water balance there was reaccumulation of both fractions. When the supraoptico-hypophysial tract was interrupted in dehydrated animals, there was accumulation of carrier substance and hormones in the proximal fibre stump and in the nerve cells during recovery. The distal fibre stump and posterior lobe remained empty. Bioassays of posterior pituitary tissue grown *in vitro* gave the evidence of no production of hormones. Hormones transferred with the explants are destroyed within 7 to 10 days. The neurosecretory cells which were grown *in vitro* for from 12 to 68 days contained small amount of neurosecretory material. That there was an evidence of peripherally directed transportation of substances within



the axoplasm of the axons of neurosecretory neurons was proved by time lapse microcinematography (phase contrast). These observations prove that the antidiuretic, vasopressor and oxytocic hormones are not produced in the posterior pituitary but they are formed in the neurosecretory nerve cells of the supraoptic and paraventricular nuclei and transported along the supraoptico-hypophysial tract to the posterior lobe where they are stored and released on demand. The parallelism between the neurosecretory material and the hormone content in the posterior lobe was also confirmed by Adamsons *et al.* (1956), Diamond (1956), Vogt (1953), and Moreno *et al.* (1955).

The depletion of the neurosecretory material from the praesoptico-hypophysial system and the caudal neurosecretory system after changes in the osmotic stimuli speaks for the presence of one antidiuretic factor in these systems of *C. mrigala*, *Anabas testudineus* and the *Rita* fish. The neurosecretory material is replenished by transference to fresh water. The order of replenishment has also been mentioned and it is identical in both the systems. First of all the storage-release centre, then the tracts and lastly the cells are filled with neurosecretory material. Thus it is seen that both the systems take part in osmoregulation. The cells of nucleus tuberis lateralis do not show any change after alteration in osmotic stimuli. These cells are related to the reproductive cycle of the fish (Scharrer 1936 ; Hild 1950 ; Stahl 1957).

#### (d) Summary :

Depletion of NSM in the praesoptico-hypophysial system and the caudal neurosecretory system has been observed after changing the osmotic stimuli in *C. mrigala*, *Anabas testudineus* and *Rita* fish. These systems take part in osmoregulation. The cells of the nucleus tuberis lateralis are not engaged in this process. They are related to the reproductive cycle.

### (B) Section Experiments

#### (a) Material and methods :

Twenty-two specimens of *C. mrigala* of 10 to 12 inches in length were used. The spinal cord was transected surgically in front of the terminal swelling which functions as a storage-release body of the caudal neurosecretory system. The specimens were examined from one day to three weeks after operation. Fixation of the tissues was done in Bouin's fluid, 10 per cent. formalin, Susa and 96 per cent alcohol. Daparaffinized sections were stained with Gomori's chrome-alum-haematoxylin-phloxin, toluidine blue, and trichrome stain after Masson.

#### (b) Results :

By surgical transection the caudal neurosecretory system was divided into two parts—the proximal one containing the neurosecretory cells and the



fibres and the distal one having some of the cells, the neurosecretory fibres and the terminal swelling (the storage-release body).

The neurosecretory cells have got good resistant power after surgical transection. The cells in the immediate vicinity of the lesion show degenerative change but more proximally the cells are intact and show hyperfunction. The degenerated cells show pyknosis of the nucleus, very scanty neurosecretory material in the form of granules, and the cytoplasm takes a deep stain (Pl. LIV, Figs. 39, 40). The cells in the distal part showed normal and degenerated forms. The cells looking normal were not functioning properly otherwise the storage body would not atrophy and it would have been filled up with neurosecretory material. The terminal storage organ is atrophic after section, with small lumina appearing in it (Pl. LV, Fig. 41). Very scanty neurosecretory material is noted.

Accumulation of neurosecretory material is noted in the proximal stump within 48 h (Pl. LV, Fig. 42). Then gradually the accumulation increases and it surrounds newly-formed capillaries. Ultimately a new depot organization is formed. The neurosecretory fibres contain neurosecretory material in the proximal stump. That the accumulated neurosecretory material at the end of the proximal stump diminishes after changes in osmotic stimulation has been mentioned previously.

#### (c) Discussion :

Hild (1951) found that the proximal stump of the cut pituitary stalk was filled with neurosecretory material 65 h after section. Sano and Hartmann (1959) found the accumulation of neurosecretory material in the proximal stump of sectioned caudal spinal cord of *Tinca vulgaris* after 48 h. Inoue (1959) found some accumulation of the neurosecretory material in the proximal stump of sectioned caudal spinal cord of *Anguilla japonica* after eight days and the maximum accumulation of the secretory material was noted in the specimen 32 days after operation. Accumulation of NSM in the proximal stump of the interrupted caudal spinal cord of *C. mrigala* is first noted after 48 h. Then gradually the accumulation increases and ultimately a new depot organization is formed. Such type of accumulation of neurosecretory material in the proximal stump of interrupted tractus hypophyseus was observed by many in different vertebrates (Hild 1951 ; Palay 1953 ; Scharrer and Wittenstein 1952 ; Roy 1957 ; and others). Roy (1960a) observed the appearance of neurosecretory material in the proximal stump of interrupted pituitary stalk of guinea pigs after two weeks and this was in excess after one-month. At both periods the neurosecretory material from the stump diminished in response to stress.

Billenstein and Leveque (1955) studied the reorganization of the neurohypophysial stalk following hypophysectomy in the rat. Two months after hypophysectomy the reorganized end of the stalk had the histological appearance of normal neural lobe. Barnett (1954) found increased disulphide groups in the infundibular stalk in the hypophysectomized rat. Lloyd and Pierog (1955) found increased ADH content in the median eminence of the hypophysectomized rat. Billenstein and Leveque (1955) state that the administration of adrenal cortical substitution therapy plus dehydration caused the



reorganized neurohypophysis to be depleted of neurosecretory material. In the present investigation (as stated previously) the accumulated NSM in the proximal stump of the interrupted caudal spinal cord in *C. mrigala* diminishes in response to the changes in osmotic stimuli.

Roy (1960a) found the median eminence of stalk sectioned dogs to be enlarged when compared to that in control animals. Campbell and Harris (1957) noted hypertrophy of the median eminence in the stalk sectioned rabbits. Magoun *et al.* (1939) mentioned an apparent hypertrophy of the median eminence in the stalk sectioned monkeys. Median eminence hypertrophy has been mentioned by Benson and Cowie (1956) in the partially or completely hypophysectomized rats. Increased activity in the proximal stump and in areas even more proximal to it has been observed by many (Sato, 1928 ; Lloyd and Pierog, 1955 ; Moreno *et al.*, 1955 ; Barnett, 1954 ; Billenstein and Leveque, 1955). Roy (1960a) thought that the enlarged median eminence and proximal hypophysial stump in stalk sectioned dogs is due to an accumulation of NSM which cannot pass to the hypophysis along its well-established routes. It was also observed (Roy, 1960a) that when hypothalamic extract from hypophysectomized (1st day) dogs was injected into the carotid artery of the assay animals, the percentage rise in adrenal venous 17-OHCS output was 332 per cent. as opposed to 74.1 per cent. rise in 17-OHCS output in the assay dogs when hypothalamic extracts were taken from normal dogs and injected into the exteriorized carotid artery of the assay animals. This proves increased activity of the hypothalamus and median eminence region after hypophysectomy.

In the invertebrates reorganization of storage depot after interruption of the neurosecretory path has also been noted by Passano (1951), Bliss and Welsh (1952) and others. Roy (1958) found in the *Palaemon carcinus* that when the sinus gland is removed there is accumulation of neurosecretory substance in the proximal stump.

From the present investigation it is also noted that the NSM is produced in the caudal neurosecretory cells, transported along the axons and stored in the terminal swelling and discharged whenever necessary.

#### (d) Summary :

The caudal neurosecretory system of *C. mrigala* was studied on different days after sectioning the spinal cord. Accumulation of NSM in the proximal part was noted after 48 h. This gave rise to a new depot organization subsequently. This proves the proximodistal migration of the neurosecretory material along the axons and its store in the terminal swelling. The terminal swelling became atrophic and porous with scanty neurosecretory substance. The newly-formed depot organization can respond to stress.

#### (C) Zinc, Histamine, Substance P and Corticotrophin-releasing Factor (CRF) in the Caudal Spinal Cord (Caudal Neurosecretory System).

**Zinc.**—Enami (1958a, 1959) found that zinc is closely associated with the secretory material of the caudal neurosecretory system in the eel. Both



soluble and insoluble forms of zinc have been detected but the amount of the soluble form is in excess. He states: 'The secretory material within the perikaryon, unlike that in the axon and in the end-bulb, exists in combination with ethanol insoluble zinc compounds.'

By a paper chromatographic study (with n-butanol-acetic acid-water, 40 : 10 : 50) of the crude extract of eel caudal spinal cord, Enami (1958b) came to the conclusion that the increased buoyancy effect (in tailless and finless goldfish) of the eluate from the topmost section of the paper chromatographic development (Rf. 0.25) was due to its zinc ingredient. The actual hormonal principle was contained in a lower fraction of the paper chromatogram and this was responsible for the second buoyancy effect without affecting the respiration.

Enami (1959) tried to isolate the effective fraction by :

- (a) removal of lipids,
- (b) precipitation of the active principle with 85 per cent. acetone,
- (c) fractionation with 45 per cent. ethanol, and
- (d) further fractionation by paper electrophoresis.

Fractions A and A 1 at pH 7.8 in the paper electrophoresis showed no characteristic dithizone-positive reaction. In the paper electrophoresis of fraction A 2 at pH 3.0, a pronounced reaction was noted midway between A 3 and A 4 which were ninhydrin-positive bands. However, when fractions A and A 1 were subjected to paper chromatography in n-butanol-acetic acid-water (40 : 10 : 50) at pH 2.5, a dithizone-positive band was found at Rf. 0.25. Therefore the metal forms a complex with the caudal neurosecretory hormone in such a way that it is separable in acid media.

Roy (1961) found that zinc is an important stimulant for the hypothalamo-pituitary-adrenal axis and it was also thought that the secretion of the caudal neurosecretory system might have some action on the said axis.

**Histamine.**—Histamine is present in the diencephalon and the caudal spinal cord of fish (Pl. LV, Fig. 43). Histamine liberated in the median eminence is carried through the hypophysis-portal vessels into the anterior pituitary and stimulates ACTH release (Harris *et al.*, 1952 ; Roy, 1960a).

**Substance P.**—Substance P is a very good smooth muscle stimulating factor. This is present in the hypothalamus (Pernor, 1953 ; Gaddum, 1960). A fraction of substance P from gut stimulates ACTH release *in vitro* (Guillemin *et al.*, 1957). Substance P has no ACTH-releasing activity (adrenal ascorbic acid depletion method) *in vivo* (Martini 1958 ; Casentini *et al.* 1959). Sedating and tranquillizing action (Stern and Milin, 1959) are noted after injection of substance P.

Euler and Östlund (1958) found the presence of substance P in the brain of the cod fish (*Gadus callarias*), the ray (*Raja batis*) and the hag fish (*Myxine glutinosa*) in amounts of 3.3 to 8.5 units per g. Large amounts of substance P were found in the spinal cord of *Raja batis* (19-36 units per g).



Further investigations by spectrographic method showed that zinc is present in the diencephalon and in the caudal spinal cord of *L. rohita*, *C. catla* and *C. mrigala*. Both soluble and insoluble forms of zinc are present in the caudal spinal cord.

Crude spinal cord extracts with water, acid, alcohol and acetone had stimulating action on the pituitary-adrenal axis of the fish, guinea pigs and dogs. The crude extracts will contain zinc, histamine and other substances which have a modifying influence over the pituitary-adrenal axis. In order to clarify this point watery extract of the caudal spinal cord including the terminal swelling was subjected to paper chromatography (in n-butanol-acetic acid-water, 40 : 10 : 50) for 6 to 8 h. When the chromatogram was developed in ninhydrin, several spots of greater or lesser intensity were noted (Rf. 0.05, 0.17, 0.25, 0.45, 0.73, 0.91). Spots at Rf. 0.05, 0.17 and 0.25 are clear. The spot at Rf. 0.25 shows maximum intensity. Spots at Rf. 0.45, 0.73 and 0.91 are indistinct ones. Zinc sulphate shows dithizone-positive spot at Rf. 0.25. One of the previous strips of paper chromatogram was developed with dithizone and that showed positive spot at about Rf. 0.25. Thus the fraction at about Rf. 0.25 is mixed with zinc. This observation is in full agreement with that of Enami (1959).

Next the adjacent strips were cut and eluted with water and tested for action on the pituitary-adrenal axis of the fish, guinea pigs and dogs. Table 1 shows the response.

TABLE 1

Rf.	Response in the form of increased plasma 17-hydroxycorticosteroids in the fish and guinea pigs and adrenal venous blood 17-hydroxycorticosteroids output (gamma/min) in dogs.	
0.05	..	Less response
0.17	..	Good response
0.25	..	" "
0.45	..	Less response
0.73		
0.91		

When the crude watery extract of the caudal spinal cord of the above-mentioned types of fishes was subjected to ascending chromatography in solvent system 1 of Guillemain *et al.* (1957), ninhydrin-positive spots appeared between Rf. 0.62 and 0.95. Corresponding areas of adjacent strips were eluted with water and tested for action on the pituitary-adrenal axis. Good response was achieved at about Rf. 0.92.

Alcoholic extract of caudal spinal cord including the terminal swelling was subjected to paper electrophoresis in phosphate buffer of pH 7.8 and  $\mu=0.6$ , 7V/cm, two h. Four or five ninhydrin-positive bands were noted of which three moved towards cathode and one or two towards anode.



Eluted materials (watery) from the cathodic side had more stimulating action on the pituitary-adrenal axis than those from the anodic one. Another important observation is that the material makes the animal less agile and quiet (Pls. LV, LVI, Figs. 44, 45). These actions simulate central actions of substance P. When the material from the caudal spinal cord was prepared and purified after the method of Guillemin *et al.* (1957) and subjected to paper electro-phoresis as mentioned above, similar findings were noted regarding the activity of the pituitary-adrenal axis. There was no variation in the findings when the starting materials were taken either from *L. rohita*, *C. mrigala* or *C. catla*.

After the above-mentioned findings were noted, it was next decided whether a corticotrophin-releasing factor (CRF) can be isolated from the caudal spinal cord (caudal neurosecretory system) of *L. rohita*, *C. mrigala* and *C. catla*. The methods of preparation of the extract and the findings will be given only for *L. rohita* as the findings are similar in the three cases.

#### (a) *Material and methods*

The starting material was the terminal part of the caudal spinal cord of *L. rohita* including the terminal swelling (caudal neurosecretory system). For control studies more proximal parts of the spinal cords were taken. The extraction procedure and the purification of the active fraction was after the method of Guillemin *et al.* (1957). The extracts were injected (intra-carotid) into the assay animals (male dogs—10 to 15 kg in weight). The assay animals have been prepared by exteriorization of the carotid artery and the animals have been conditioned for intracarotid injections. Cannulation of the right adrenal vein has been performed before the experiments. Blood was collected 30 min after injection of the extracts. Hypophysectomy was done by the transtemporal route and it was thought to be complete when no pituitary tissue was found in the pituitary fossa or in areas nearby in histological sections. The determination of plasma 17-hydroxycorticosteroids was done after the method of Silber and Porter (1954).

The extracts were also injected intraperitoneally into *L. rohita* (10 to 12 inches in length). Three hours after, the tail was severed and the blood was collected in a heparinized pot. The plasma was separated and 17-hydroxycorticosteroids were estimated after the method of Silber and Porter (1954).

The pituitaries of the dogs have been fixed in 10 per cent formaldehyde and paraffin sections have been cut and stained by haematoxylin and eosin and Masson's trichrome stain. The adrenals of the dogs were fixed in 10 per cent formaldehyde and frozen sections stained with sudan III and paraffin sections stained by haematoxylin and eosin.

#### (b) *Results :*

In solvent system 1 of Guillemin *et al.* (1957), the caudal spinal cord extract showed two main spots between Rf. 0.68 and 0.94. The most potent fraction for the stimulation of the pituitary-adrenal axis is that one with Rf. 0.94. At smaller doses maximum stimulation was noted. At higher doses there was depression (Tables 2 and 3). This material is ninhydrin



positive and is peptide. From the proximal spinal cord the material had some stimulatory action but not to the extent as observed after caudal neurosecretory extract. Presence of the pituitary in the assay animals is essential for the mediation of the response.

The extract from the caudal spinal cord (Rf. 0.94) had no vasopressor activity (Pl. LVI, Fig. 46). This fraction had no activity on the guinea pig's isolated terminal ileum and it did not contain any antidiuretic activity. It had also no oxytocic activity.

As a preliminary report it can be mentioned that a material with Rf. 0.9 can also be prepared from the diencephalon of *L. rohita* having a stimulatory action on the pituitary-adrenal axis.

Table 2 shows the activity of the extracts (Rf. 0.9) and control extracts (from proximal spinal cord) on the pituitary-adrenal axis of the assay dogs.

TABLE 2

Dose of the extract injected into the carotid artery ( $\mu$ g)	Adrenal venous 17-hydroxycortico- steroids output ( $\mu$ g/min)	
	Before injection	After injection
(Average of 5 observations)		
Control extracts (50) .. ..	2.3	3.2
Control extracts (100) .. ..	1.8	5.9
Caudal spinal cord extracts (Rf. 0.9) (50) ..	1.7	13.3
Caudal spinal cord extracts (Rf. 0.9) (100) ..	2.1	3.8

Same extracts injected into hypophysectomized assay dogs (48 h after hypophysectomy) had no stimulatory action.

The extracts were also injected into ventral hypothalamectomized dogs and dogs with isolated pituitary preparations (Roy, 1960b). In both instances

TABLE 3

*Plasma 17-hydroxycorticosteroids of L. rohita after injection of the extracts (Rf. 0.9 and control extracts (from proximal spinal cord))*

Dose of the extracts ( $\mu$ g)	Plasma 17-hydroxycorticosteroids in $\mu$ g/100 ml (average of 5 animals)
No treatment .. ..	17.5, 16.2, 19.3, 11.0, 13.4=15.4
Control extracts (5) .. ..	10.6, 18.2, 22.5, 19.6, 21.8=18.5
Control extracts (10) .. ..	20.2, 18.3, 24.6, 22.5, 17.3=20.5
Caudal spinal cord extracts (Rf. 0.9) (5) ..	45.3, 54.5, 42.3, 38.2, 62.1=48.4
Caudal spinal cord extracts (Rf. 0.9) (10) ..	16.5, 12.3, 18.6, 21.2, 13.3=16.3



rise in adrenal venous blood 17-OHCS output was noted with the caudal neurosecretory extract (Rf. 0.9) and central (diencephalic) neurosecretory extract.

The adrenals of the dogs with high 17-OHCS output showed depletion of the sudanophilic material from the fascicular zone and cytolytic and tubular changes in the same zone (Pl. LVI, Fig. 47) indicating hyperactivity of the gland. The pituitary gland showed increased number of basophil cells with vacuolar change (Pl. LVI, Fig. 48).

### (c) Discussion :

In the present investigation, the extract from the caudal spinal cord of the fish gives rise to two ninhydrin-positive spots between Rf. 0.68 and 0.94 in solvent 1. In the same solvent system, Guillemin *et al.* (1957) found four fractions, A, B, C, D with mean Rf. values 0.06, 0.4, 0.7, 0.9 respectively from hypothalamic extracts of pork and beef. The differences may be due to the type of animals or the type of tissues extracted. The fraction about Rf. 0.9 is an important stimulus for ACTH release in both the conditions. McCann (1957) found that the fraction with Rf. 0.8 to 1.0 was ineffective in ACTH discharge, even when injected at a higher dose and this fraction virtually had no pressor activity. Both ACTH-releasing activity and pressor activity were found in the same portion of the chromatogram with Rf. 0.3 to 0.8. Presence of the pituitary in the assay animals is essential for the response. More proximal part of the spinal cord did not contain this material. The fraction about Rf. 0.9 is a peptide and it does not contain histamine or any vasopressor, antidiuretic or oxytocic activity.

The active fraction after its release from the caudal neurosecretory system reaches the pituitary through the systemic circulation to act on it. It is a surprise why the material is to traverse such a long distance for its action. But Guillemin *et al.* (1957) state : 'The ACTH-releasing principle would be a particular substance or structure with a wide distribution pattern in the organism... The ACTH-hypophysiotropic material of extra-hypothalamic origin released from various tissues by a non-specific stress and delivered through the general circulation would explain the release of corticotrophin which can still take place under severe nonspecific stress after severance of the normal hypothalamo-pituitary relationship or destruction of median eminence.' Morphological changes in the adrenals and the pituitaries evidenced hyperactivity.

### (d) Summary and conclusion :

From the caudal spinal cord including the terminal swelling (caudal neurosecretory system) of the Indian teleost fish *L. rohita*, a material (peptide) has been isolated which stimulates the release of ACTH *in vivo*. This does not contain histamine or any vasopressor, antidiuretic or oxytocic activity.



We conclude this study of the caudal neurosecretory system of some of the Indian fishes by comparing and contrasting this system with the hypothalamo-neurohypophysial system :

Hypothalamo-neurohypophysial system	Caudal neurosecretory system
1. Neurosecretory cell station in the hypothalamus. Neurosecretory pathway known as the supraoptico or praesoptico-hypophysial tract. Neurohypophysis functions as the storage organ. This system develops earlier.	1. Neurosecretory cell station in the caudal spinal cord with a caudal neurosecretory pathway. The terminal swelling functions as the storage organ. This system develops later.
2. The neurosecretory cells and the neurohypophysis are highly vascular.	2. The neurosecretory cells and the terminal swelling of the spinal cord are highly vascular.
3. Neurosecretory material has been noted in the vessels of the neurohypophysis.	3. Neurosecretory material has been noted in the vessels of the terminal swelling.
4. Infundibular recess of the third ventricle is seen near the neurohypophysis.	4. Usually such type of dilatation of the central canal near the terminal swelling is not found except in <i>Wallago attu</i> .
5. The neurosecretory material is chrome-alum-haematoxylinophilic.	5. The neurosecretory material is phloxinophilic.
6. Beaded appearance along the axon is noted (neurosecretory beads).	6. Beaded appearance along the axon is noted (neurosecretory beads).
7. Herring bodies and pituicytes are present in the neurohypophysis.	7. Herring body-like structures and pituicyte-like structures are present in the terminal swelling.
8. Neurosecretory material comes along the neurosecretory path and accumulates surrounding the blood-vessels in the neurohypophysis.	8. Neurosecretory material comes along the neurosecretory path and accumulates surrounding the blood-vessels in the terminal swelling.
9. Secretion cycle is noted in the neurosecretory cells.	9. Secretion cycle is noted in the neurosecretory cells.
10. This system responds to osmotic stimuli.	10. This system responds to osmotic stimuli.
11. Responds to stress.	11. Responds to stress.
12. The neurosecretory material is glycolipoprotein in nature.	12. The neurosecretory material is carbohydrate-protein in nature.
13. Section experiment of the pituitary stalk leads to accumulation of the neurosecretory material in the proximal stump. This proves proximodistal migration of the neurosecretory material.	13. Section experiment of the caudal spinal cord in front of the terminal swelling leads to accumulation of the neurosecretory material in the proximal stump. This proves proximodistal migration of the neurosecretory material.
14. There is neoformation of neurohypophysis-like tissue in the proximal stump after hypophysectomy. This tissue responds to stress by depletion of neurosecretory material.	14. There is neoformation of the terminal swelling in the proximal stump after cord section. This newly-formed tissue responds to stress by depletion of neurosecretory material.



Hypothalamo-neurohypophysial system	Caudal neurosecretory system
15. The neurohypophysis atrophies after stalk section.	15. The original terminal swelling atrophies after cord section.
16. The neurosecretory cells of the hypothalamus have got great resistant power as they survive after stalk section and produce more neurosecretory material due to injury stimulus.	16. The neurosecretory cells of the caudal neurosecretory system have got great resistant power as they survive after cord section and produce more neurosecretory material due to injury stimulus.
17. The neurosecretory cells produce vasopressin, antidiuretic and oxytocic hormones.	17. The neurosecretory cells have got the following functions : (a) They take part in sodium exchange. (b) They have got relation to gas metabolism. (c) They have got action on gill morphology.
18. Corticotrophin-releasing afctor (CRF) has been isolated.	18. Corticotrophin-releasing factor (CRF) has been isolated.

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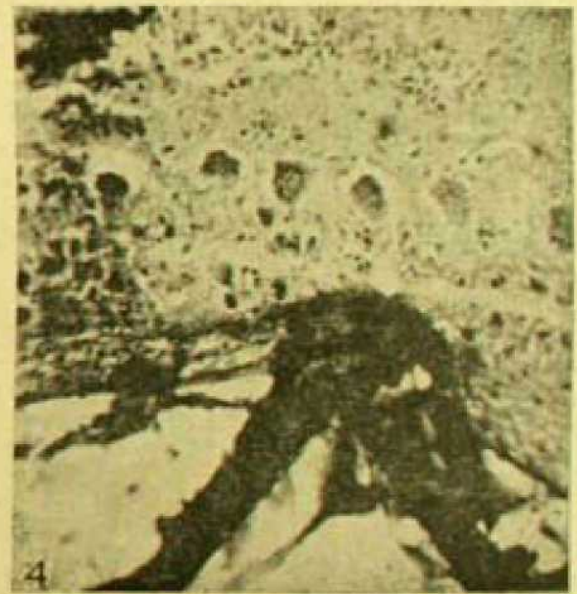
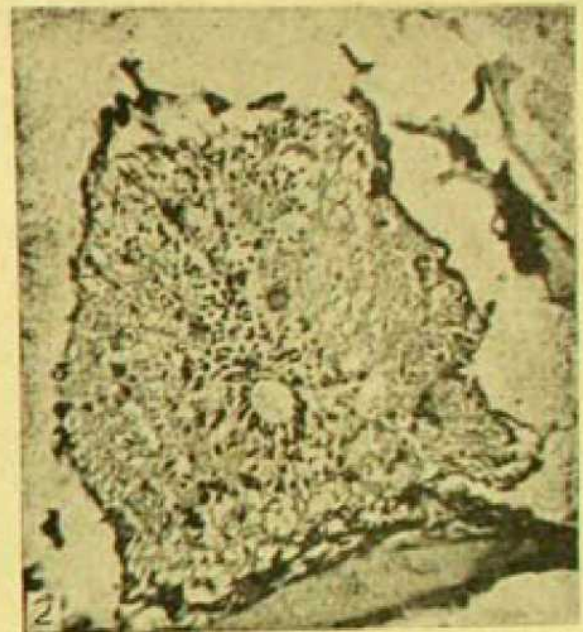
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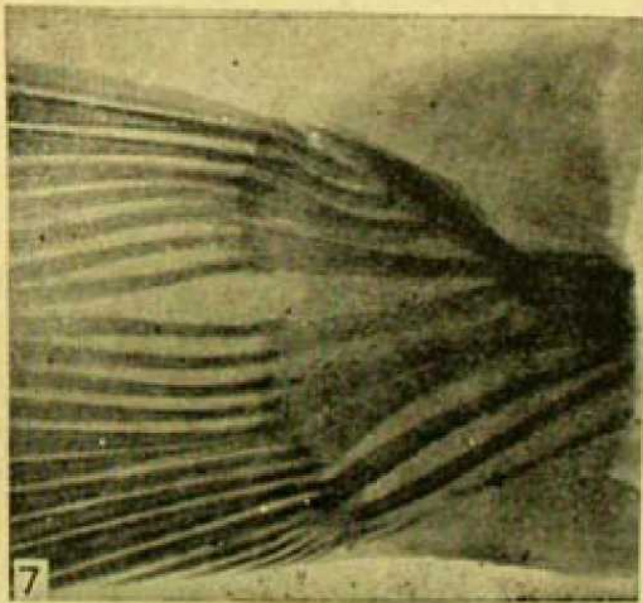
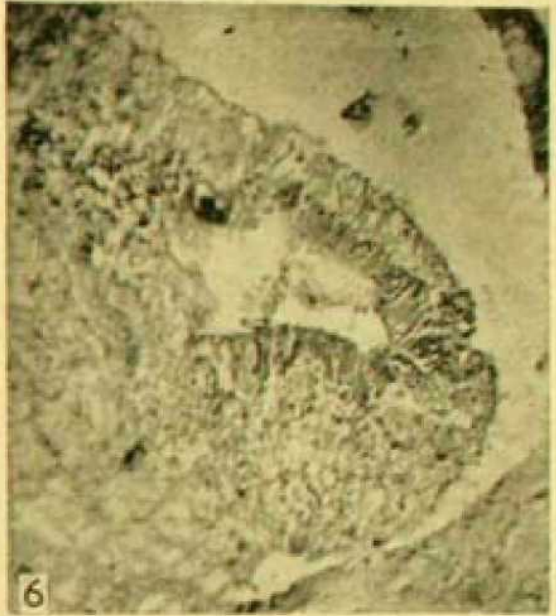
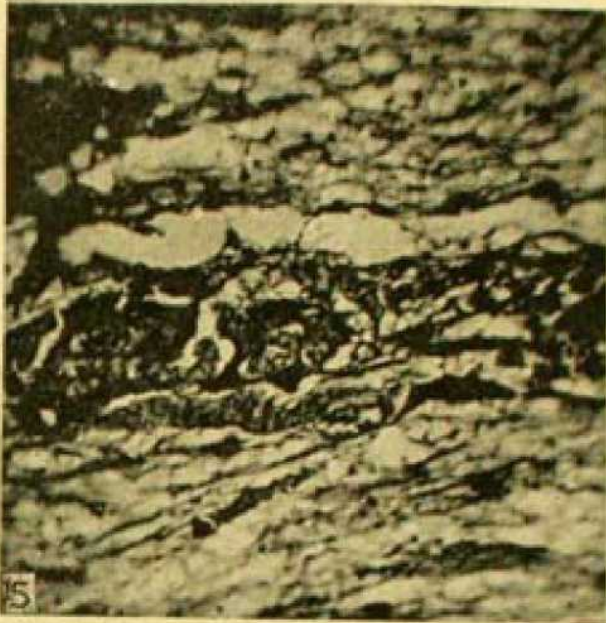


## Chapter—6



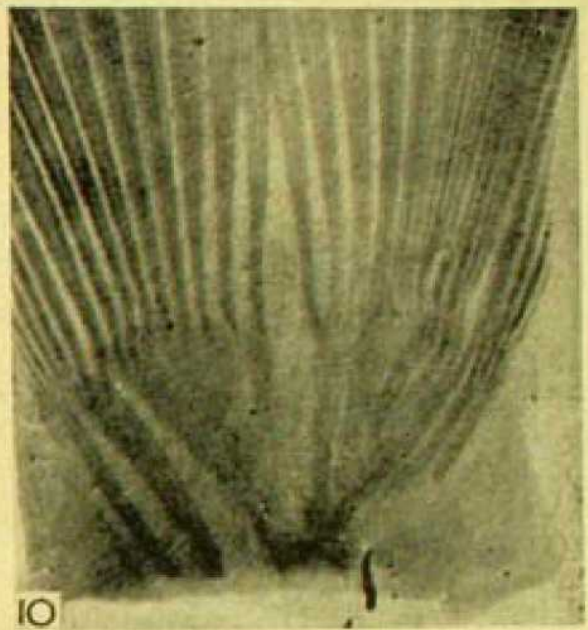
- FIG. 1. Caudal part of the spinal cord of the larva (two weeks) of *L. rohita* showing ventral herniation of the part. The interval between this and the chorda tissue is occupied by a capillary net. Mallory's stain.  $\times 215$ .
- FIG. 2. Transverse section through the caudal part of the spinal cord of the larva (two weeks) of *L. rohita* showing the deficiency of the meningeal covering between the cord and the chorda tissue. The space is occupied by a leash of blood-vessels. Above this there is preponderance of small cells. Mallory's triple stain.  $\times 215$ .
- FIG. 3. Groups of praectopic cells in the larva (two weeks) of *L. rohita*. Gomori's chrome-alum-haematoxylin-phloxin stain.  $\times 215$ .
- FIG. 4. Spinal cord neurons of the larva (two weeks) of *C. catla*. Mallory's stain.  $\times 215$ .





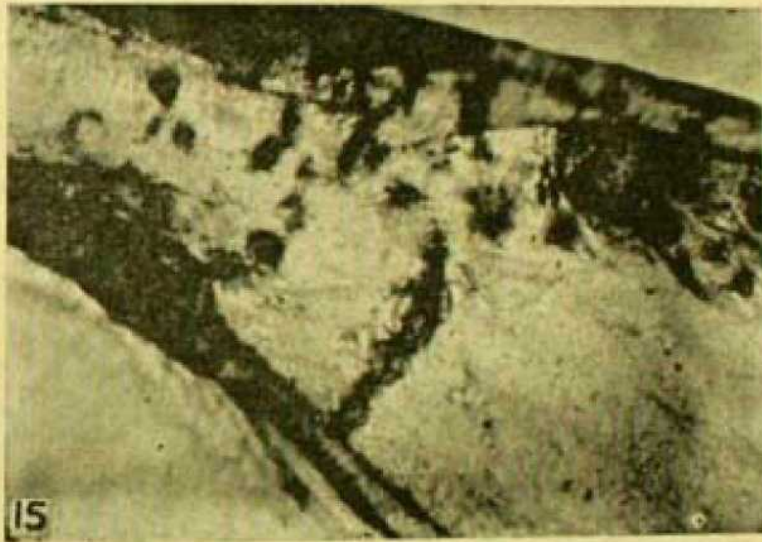
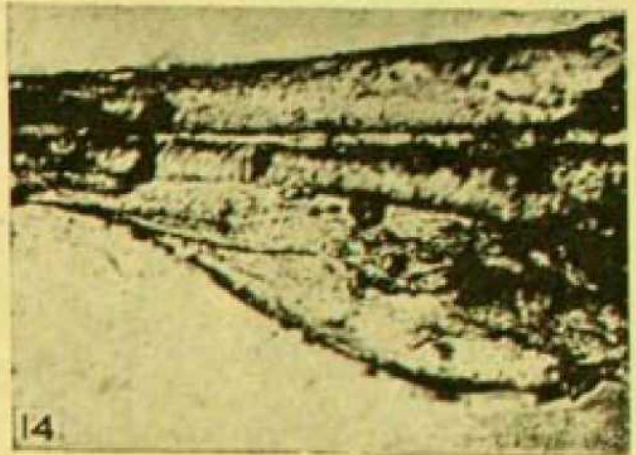
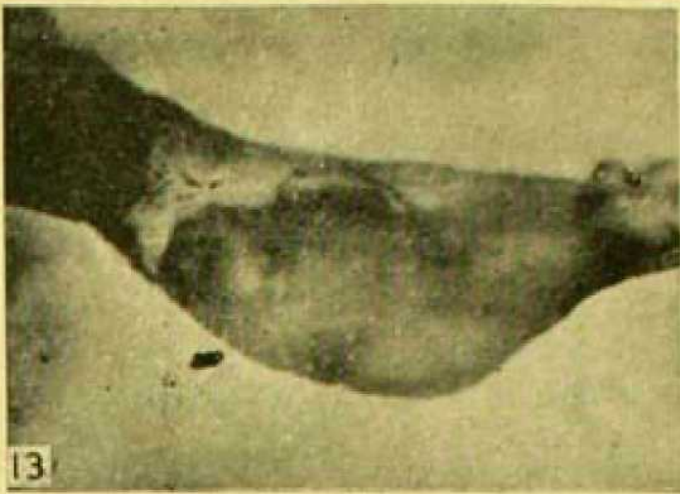
- FIG. 5. Endo- and pericellular blood-vessels of the caudal neurosecretory cells of *Mugil tade* Mallory's stain.  $\times 215$ .
- FIG. 6. Shows expansion of the central canal in the terminal swelling of *Wallago attu*. Mallory's stain.  $\times 215$ .
- FIG. 7. Radiograph showing the exact location of the terminal swelling of *L. rohita* by a pin-head.
- FIG. 8. Radiograph showing the exact location of the terminal swelling of *C. mrigala* by a pin-head.





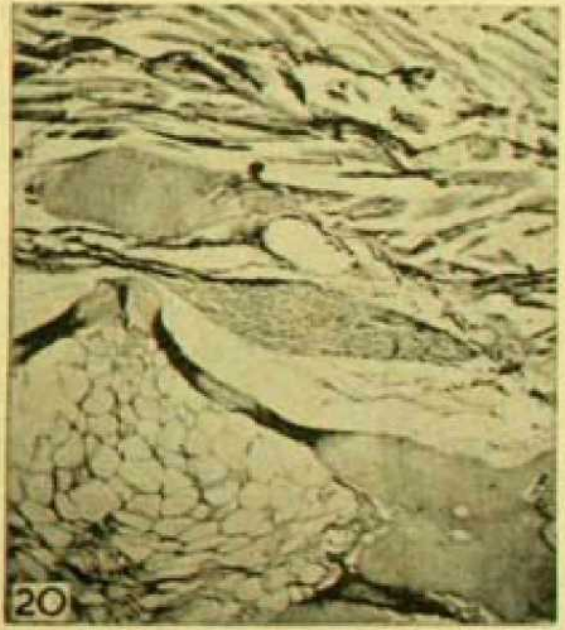
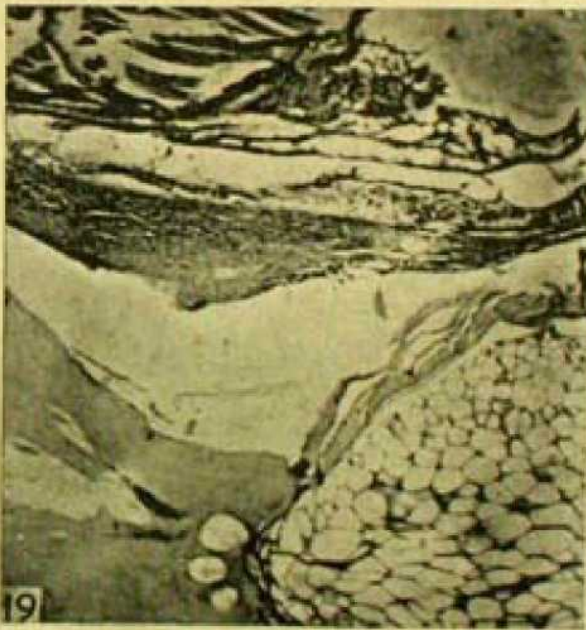
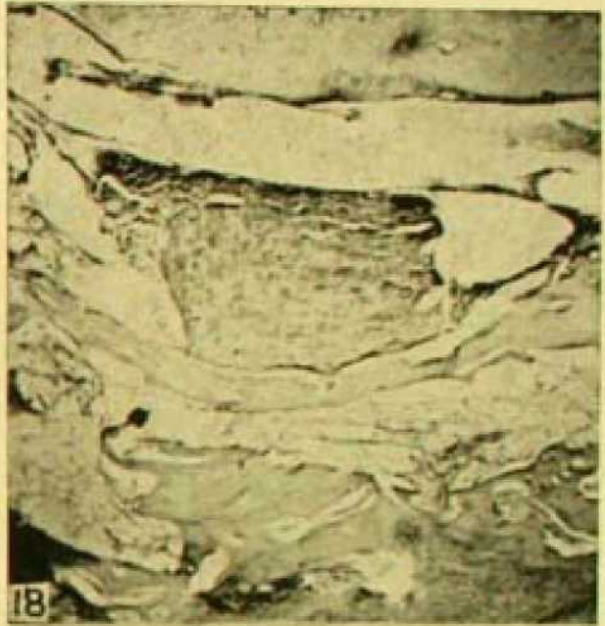
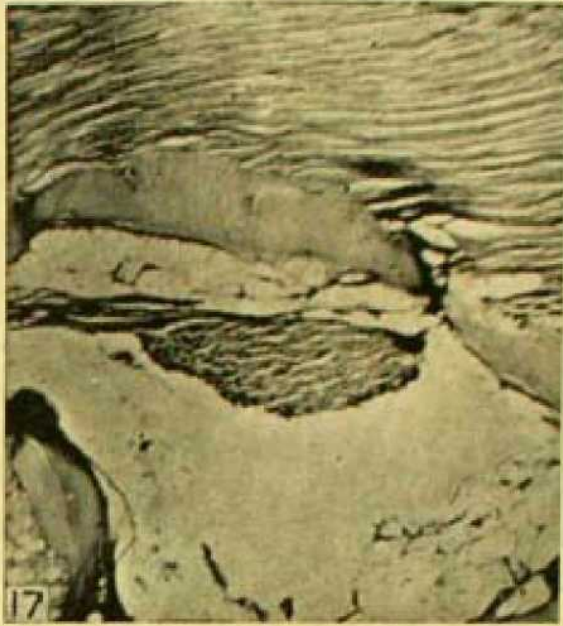
- FIG. 9. Radiograph showing the exact location of the terminal swelling of *Lates calcarifer* by a pin-head.
- FIG. 10. Radiograph showing the exact location of the terminal swelling of *O. catla* by a pin-head.
- FIG. 11. Terminal swelling of *H. ilisha*.
- FIG. 12. Terminal swelling of *O. catla*.





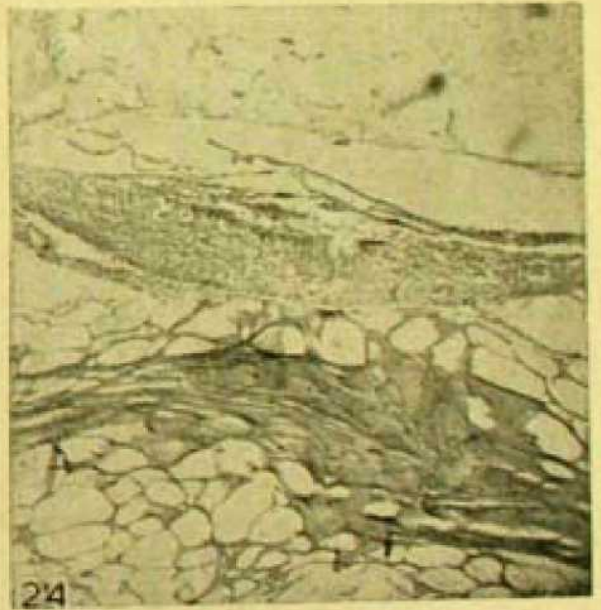
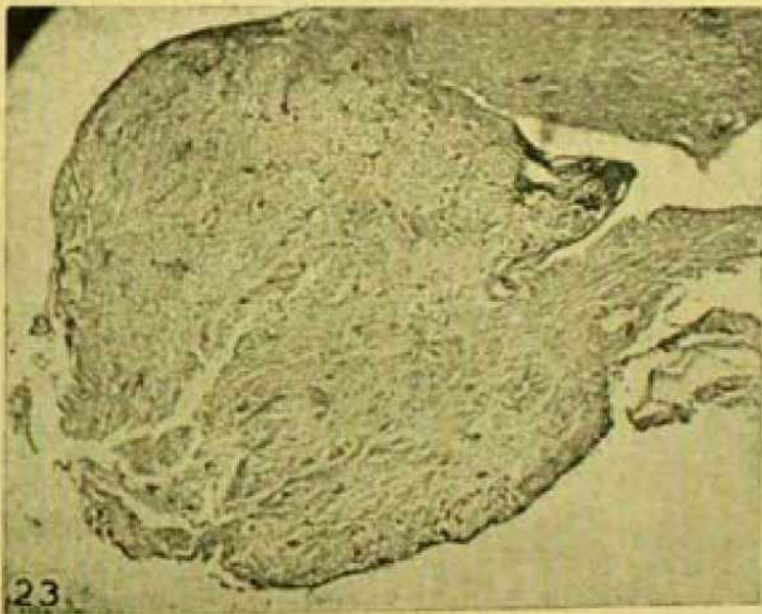
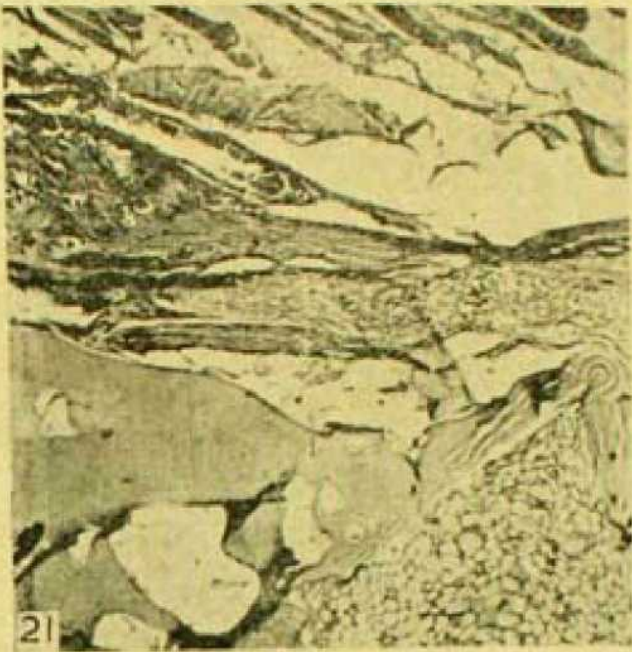
- FIG. 13. Terminal swelling of *L. rohita*.  
 FIG. 14. Terminal swelling of *L. Akalbasu*.  
 FIG. 15. Terminal swelling of *Wallago attu*.  
 FIG. 16. Terminal swelling of *O. punctatus*.





- FIG. 17. Terminal swelling of *Anabas testudineus*.  
 FIG. 18. Terminal swelling of *Ophicephalus striatus*.  
 FIG. 19. Terminal swelling of *Clarias batrachus*.  
 FIG. 20. Terminal swelling of *Heteropneustes fossilis*.





- FIG. 21. Terminal swelling of *Macroneis vitatus*.  
 FIG. 22. Terminal swelling of *Lates calcarifer*.  
 FIG. 23. Terminal swelling of *C. nrigala*.  
 FIG. 24. Terminal swelling of *Notopterus chitala*.



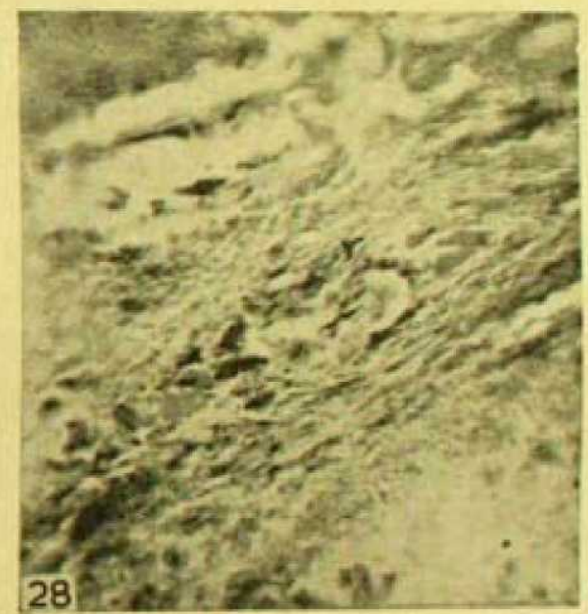
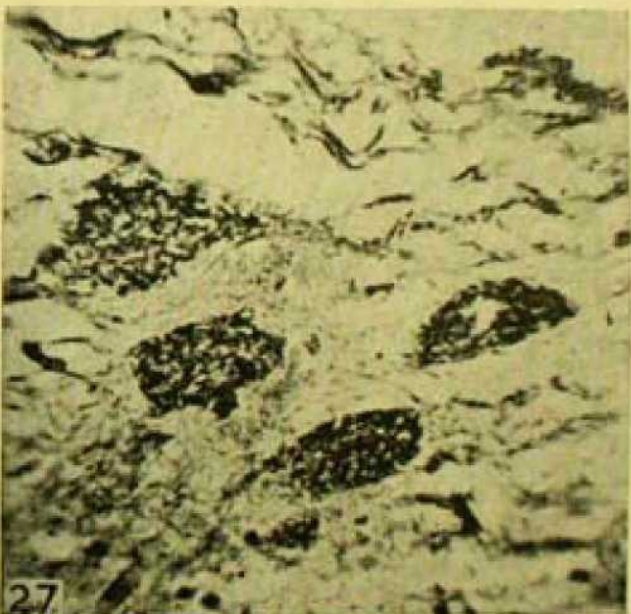
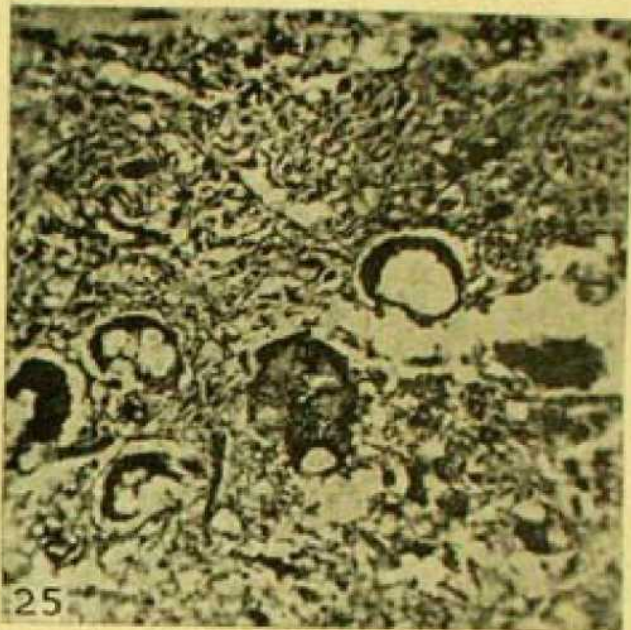


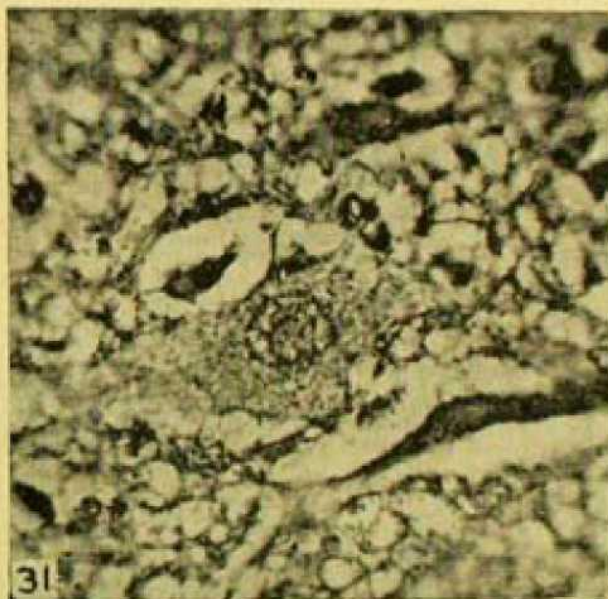
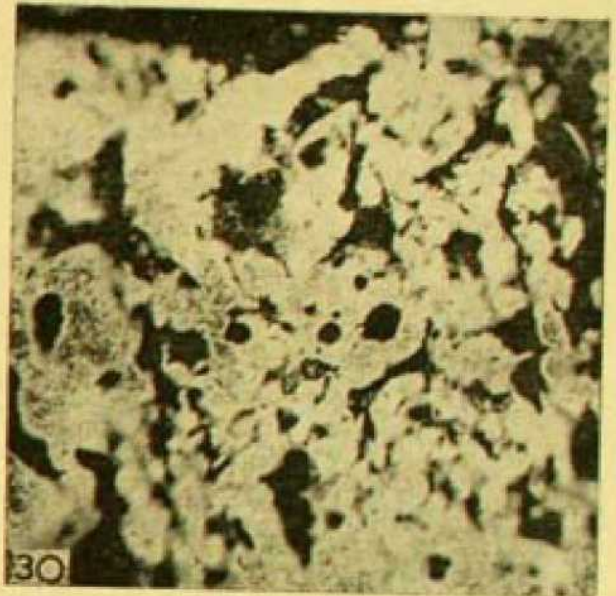
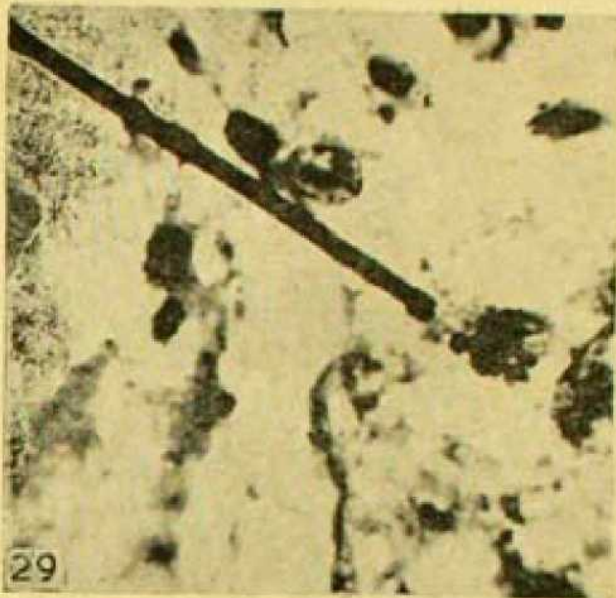
FIG. 25. Caudal neurosecretory cells of *Exocetus mento* showing neurosecretory material and vacuolated appearance. Gomori's chrome-alum-haematoxylin-phloxin stain.  $\times 215$

FIG. 26. Caudal neurosecretory cells of *L. rohita*. The nucleus of one cell appears empty. Mallory's stain.  $\times 215$ .

FIG. 27. Caudal neurosecretory cells of *Anabas testudineus* showing neurosecretory material in the nucleus and the cytoplasm. Mallory's stain.  $\times 450$ .

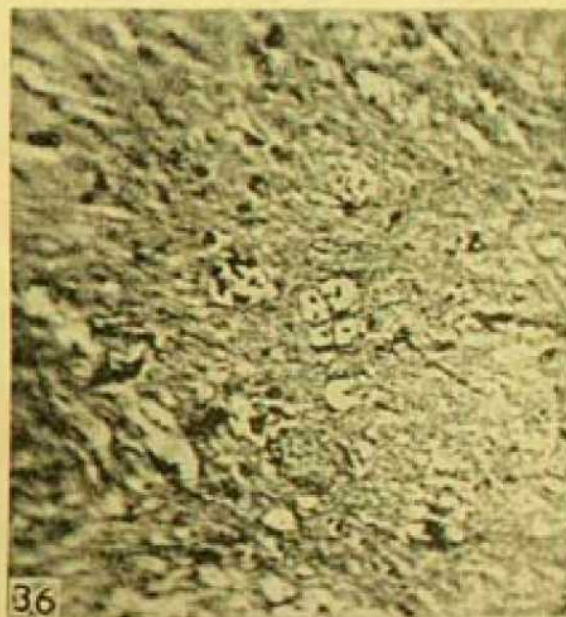
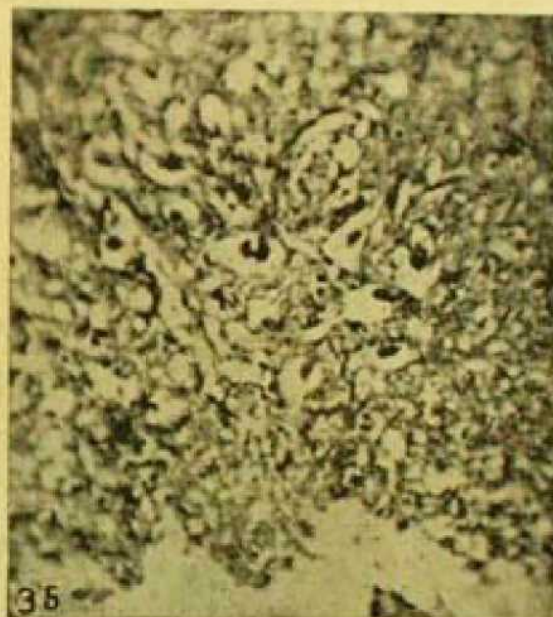
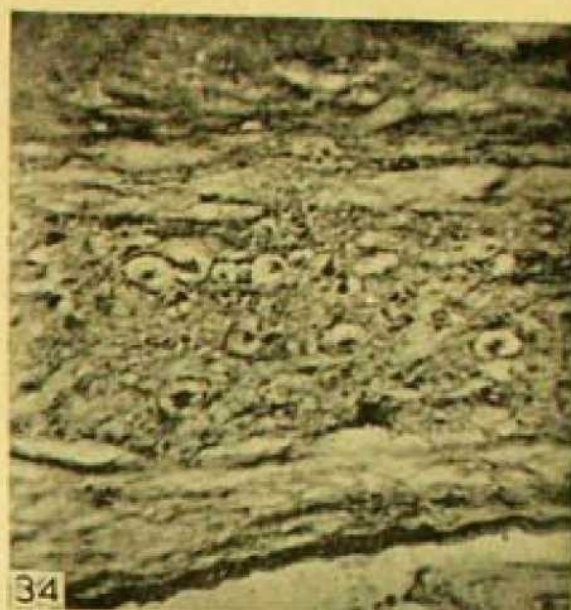
FIG. 28. Neurosecretory material is found in the lumen of a blood-vessel in the terminal swelling of *C. mrigala*. The material is also noted surrounding the vessel. Mallory's stain.  $\times 450$ .





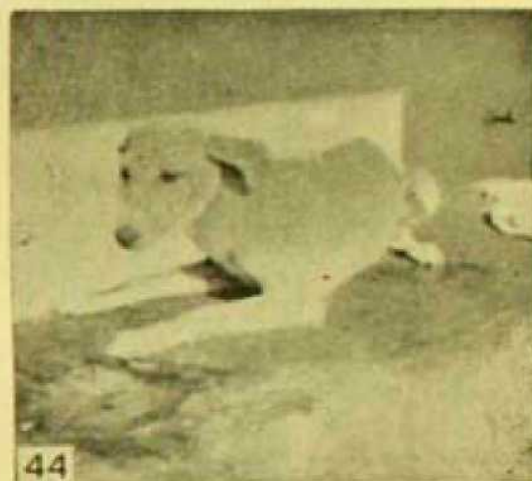
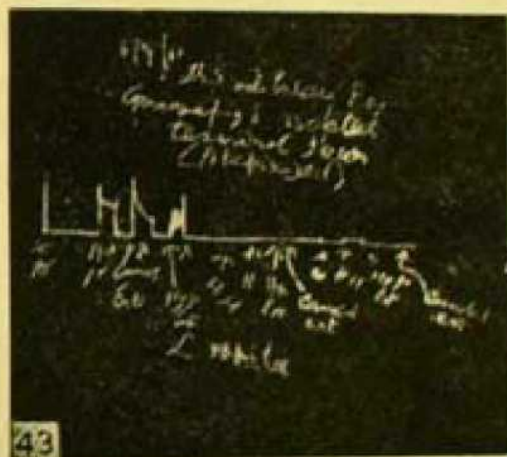
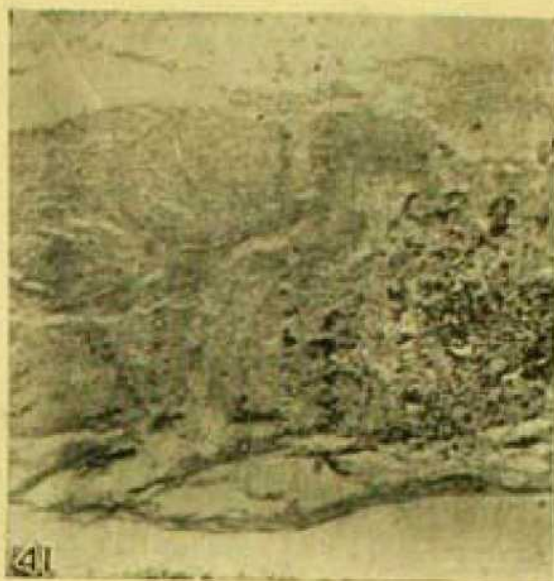
- FIG. 29. Caudal neurosecretory cells of *Trygon sephen*. Intranuclear neurosecretory material is noted. Mallory's stain.  $\times 450$ .
- FIG. 30. Caudal neurosecretory cells of *Trygon sephen* showing neurosecretory material in the nucleus and the cytoplasm. The cells also show vacuolated appearance. Mallory's stain.  $\times 215$ .
- FIG. 31. Caudal neurosecretory cells of *Trygon sephen* showing intranuclear and cytoplasmic neurosecretory material and vacuoles. Mallory's stain.  $\times 450$ .
- FIG. 32. Normal praectopic cells of *C. mrigala*. Gomori's chrome-alum-haematoxylin-phloxin stain.  $\times 215$ .





- FIG. 33. Praeoptic cells of *C. mrigala* in 1.8 per cent saline showing loss of neurosecretory material and vacuolated appearance. Gomori's chrome-alum-haematoxylin and phloxin stains.  $\times 215$ .
- FIG. 34. Caudal neurosecretory cells of *C. mrigala* in 1.8 per cent saline showing loss of neurosecretory material and vacuolated appearance. Gomori's chrome-alum-haematoxylin-phloxin stain.  $\times 215$ .
- FIG. 35. Praeoptic cells of *Anabas testudineus* in 15 per cent saline showing loss of neurosecretory material and vacuolated appearance. Gomori's chrome-alum-haematoxylin-phloxin stain.  $\times 215$ .
- FIG. 36. Praeoptic cells of *Rita* fish in 15 per cent saline showing loss of neurosecretory material and vacuolated appearance. Gomori's chrome-alum-haematoxylin-phloxin stain.  $\times 215$ .





- FIG. 41. The terminal swelling of *C. mrigala* after section of caudal spinal cord. It is atrophic, porous and there is loss of neurosecretory material. Masson's stain.  $\times 50$ .
- FIG. 42. Accumulation of neurosecretory material is noted in the proximal stump of the sectioned caudal spinal cord of *C. mrigala*. Masson's stain.  $\times 50$ .
- FIG. 43. Guinea pig's ileum. H=histamine; Caud. ext.=extract of caudal spinal cord of *L. rohita*; Hyp. e t.=hypothalamic extract of *L. rohita*; AH=six gamma anti-histamine.
- FIG. 44. Dog before injection of the extract.



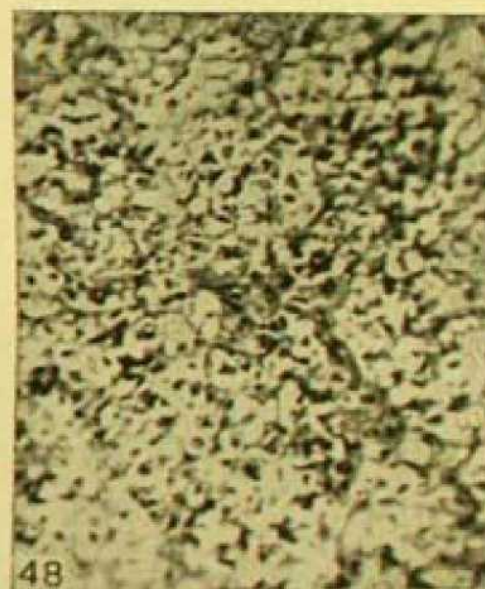
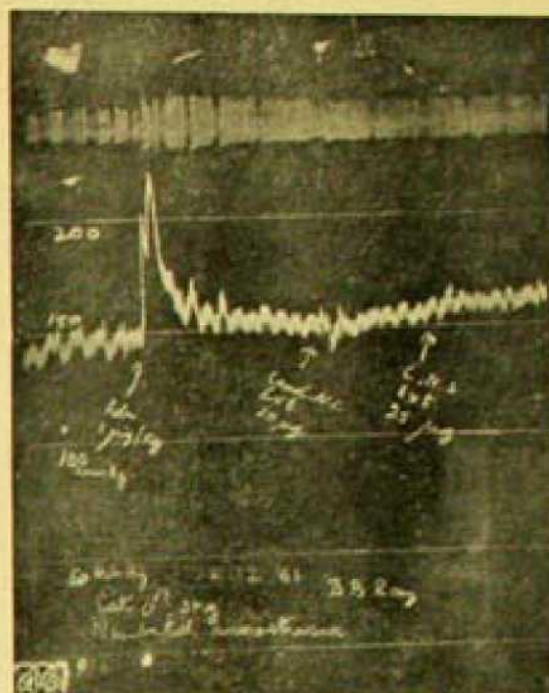
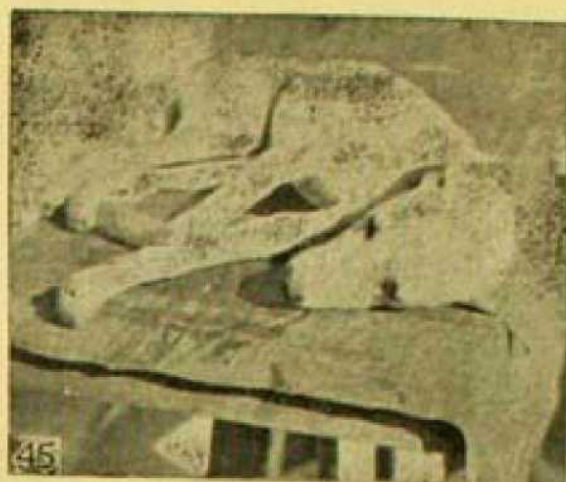


FIG. 45. Dog after injection of the extract.

FIG. 46. Blood pressure and respiration of cat after injection of the caudal neurosecretory extract showing very little change.

FIG. 47. Zona fasciculata of the adrenal of dog after injection of the extract showing cytolitic changes. Haematoxylin and eosin stain.  $\times 215$ .

FIG. 48. Anterior pituitary of the dog after injection of the extract showing vacuolated appearance of the basophil cells. Masson's stain.  $\times 215$ .



## CHAPTER—7

### ACTION OF ZINC ON THE HYPOTHALAMO-PITUITARY-ADRENAL AXIS OF THE FISH, GUINEA PIG AND DOG AND ON DIFFERENT EXPERIMENTAL PREPARATIONS 1962

#### ABSTRACT

Anterior interrenals and pituitaries of *Cirrhina mrigala*, *Catla catla* and *Labeo rohita* have been described. There is increased activity in the hypothalamo-pituitary-adrenal axis of the fish, guinea pig and dog after injection of zinc. This has been assessed by histological examination and by noting the level of plasma 17-hydroxycorticosteroid level in the fishes and guinea pigs and adrenal venous blood 17-OHCS output in dogs. ACTH leads to a rise in the level of plasma 17-OHCS level in fishes with hypertrophy of the anterior interrenal cells. For increased response of the axis after injection of zinc in guinea pigs and dogs, the pituitary is to be present. Ventral hypothalamectomy with lesion of the median eminence abolishes the response in the dog after injection of zinc due to the infarction of the pituitary. Diurnal fluctuation of 17-OHCS output disappears in dogs with surgical lesion of the hippocampus. The basal level of 17-OHCS output is increased in dogs with lesion of the hippocampus or with pituitary-island preparation. In such experimental preparations zinc leads to further increase in 17-OHCS output.

There is no change in the corpuscles of Stannius after injection of zinc or ACTH in fishes. The corpuscles may be engaged in osmoregulation or the hormones may act on the reproductive system.

Control of ACTH secretion in fishes has been discussed and the importance of the neurosecretory system (central and caudal) has been stressed.

The importance of the limbic forebrain-midbrain circuit (Nauta) has been discussed. The ponto-cerebello-medullary complex stimulates the anterior pituitary by discharge of neurosecretory substance which activates the anterior pituitary cells through systemic circulation and there is increased secretion of ACTH in response to neural or humoral stimuli.

Underwood (1956) and Vallee (1959) reviewed the metabolic role of zinc. Owen (1960) discussed the biochemical function of some of the minor elements. In the tissues of the animal body zinc is widely distributed.

Zinc is found in the seminal fluid and the prostate gland and its deficiency in rats gives rise to testicular atrophy. Parizek (1957) found that cadmium injections destroyed the testicular tissue of rats and mice but when zinc was given simultaneously, the destructive effect of cadmium was prevented. The female genital organs do not contain any high concentration of zinc.

High concentration of zinc is found in liver, bone, choroid and iris. There is a relationship between zinc and insulin and, when insulin is crystallized from pancreas, it contains zinc which is also known to be involved in some enzyme systems. It is present in carboxypeptidases and activates aminopeptidases. According to Vallee alcohol dehydrogenase of bovine liver and lactic dehydrogenase of rabbit skeletal muscle contain zinc. Owen (1960) states : 'It appears therefore that the rate of turnover of zinc may be high in such active organs as the liver where protein synthesis and



hydrolysis are rapid, in the pancreas where protein synthesis is rapid and in the intestinal mucosa where both metabolic activity and rate of replacement of the tissue are very high. Protein synthesis is also rapid in the skin which like the intestinal mucosa is continually being rubbed off. High concentrations of zinc may indicate merely a high rate of metabolic activity involving a high rate of protein synthesis such as occurs also in the male genital tract.'

Whale corticotropin contains zinc. Zinc increases the activity of gonadotropins. Enami (1958) states that zinc is associated with the secretory product of the caudal neurosecretory system in the eel. Zinc is also found in the caudal neurosecretory system of some of the fishes of Bengal and the diencephalon is rich in zinc and this will be the subject of the next communication.

In the present investigation the importance of zinc as a stimulator of the hypothalamo-pituitary-adrenal axis has been studied in local freshwater fish and animals and in different experimental preparations of the animal. In no case of fish or guinea pigs intraperitoneal injection of zinc led to any inflammatory reaction. Sobel *et al.* (1960) injected zinc chloride intraperitoneally in either sexed guinea pigs of 300 to 350 g in weight. Six-hour urine collection and examinations by Silber-Porter reaction were done. Zinc chloride was administered twice daily at doses of 4  $\mu$ M/100 g. This dose (one 4  $\mu$ M/100 g) doubled the six-hour excretion value. Single injection seems to be without any toxic reaction.

### MATERIALS AND METHODS

*Fish.*—Three types of fish (eight of each type, two serving as controls and six for experiments) have been used. They are *Labeo rohita*, *Cirrhina mrigala* and *Catla catla* of about eleven inches in length. Intraperitoneal injection of zinc sulphate at a dose of 0.1 millimoles has been given. ACTH has been injected intraperitoneally at a dose of 0.2 I.U./g (at room temperature). Three hours after the injection the tail has been severed and blood collected in a heparinized Petri dish. Blood is immediately centrifuged and plasma is separated. Plasma 17-hydroxycorticosteroids have been estimated after the method of Silber and Porter (1954). After the animals were sacrificed, the hypothalamus, pituitary and the interrenal tissues have been examined histologically. The hypothalamus has been fixed in Bouin's fluid, paraffin sections have been cut and stained by Gomori's chromealum-haematoxylin and phloxin stain. The pituitaries have been fixed in 10 per cent. formaldehyde, Bouin's fluid and Helly's fluid and paraffin sections have been cut and stained by haematoxylin and eosin, Gomori's CAHP stain and Masson's trichrome stain. Pituitaries have also been stained by Gomori's aldehyde fuchsin stain and PAS stain technique. The anterior interrenal tissues are contained in the head kidney. The tissue has been fixed in 10 per cent. formaldehyde and paraffin sections stained by Masson's trichrome stain and haematoxylin and eosin stain. Frozen sections have been stained by Sudan IV. Tissues have been prepared for demonstration of ascorbic acid granules by silver nitrate method in sections.



*Guinea pigs.*—Ten male guinea pigs of 400 to 450 g in weight have been used, two animals serving as control. Zinc sulphate at dose of 3  $\mu$ M/100 g body weight has been injected intraperitoneally. Three hours after the injection of zinc sulphate the animals have been sacrificed by decapitation and blood collected in heparinized Petri dish. Blood is immediately centrifuged and the plasma 17-hydroxycorticosteroids have been estimated after the method of Silber and Porter (1954). The hypothalamus has been fixed in Bouin's fluid and paraffin sections stained by Gomori's CAHP stain. The pituitary is fixed in 10 per cent. formaldehyde and stained by haematoxylin and eosin and Masson's trichrome stain. The adrenals have been fixed in 10 per cent. formaldehyde. Frozen sections have been stained by Sudan IV stain and paraffin sections have been stained by haematoxylin and eosin. The adrenal glands have been prepared for demonstration of ascorbic acid granules by silver nitrate method in sections. Five hypophysectomized guinea pigs have also been injected intraperitoneally with zinc sulphate solution and the animals have been sacrificed after three hours and all the examinations have been carried on as mentioned above excepting the examination of the pituitary gland.

*Dogs.*—Male dogs of 10 to 15 kg in weight have been used in this experiment. Intravenous injection of zinc sulphate at a dose of 0.2 millimoles has been given very slowly and not in a concentrated form otherwise untoward reactions occur and sometimes the animal dies. McCance and Widdowson (1942) have studied the absorption and excretion of zinc in human subjects. Inorganic zinc salts were dissolved in a convenient volume of 0.9 per cent. NaCl. The intravenous injections have been given very slowly. Zinc sulphate has been injected intravenously into the following types of animals :

- (a) normal dogs—5 in number ;
- (b) ventral hypothalamectomized dogs with lesion of median eminence—2 in number ;
- (c) dogs with surgical lesion of hippocampus—has been done in two different stages after the method of Bard and Mountcastle (1948). A segment of the fornix was also extirpated on each side. The dogs were used 7 days after operation ;
- (d) dogs with pituitary-island preparation (Roy, 1960a)—3 in number.
- (e) hypophysectomized dogs—3 in number.

In all the preparations, the right adrenal vein has been cannulated for intermittent collection of adrenal venous blood. Blood is collected in heparinized graduated centrifuge tubes. It is immediately centrifuged and plasma separated for estimation of 17-hydroxycorticosteroids after the method of Silber and Porter (1954). The hypothalamus and pituitary (where available) and the adrenals have been fixed, sectioned and stained as described in the materials and methods for guinea pigs.

Study of plasma 17-hydroxycorticosteroids has been carried out in fish (*L. rohita*, *C. mrigala* and *C. catla*) after zinc and ACTH injection. There is rise in plasma 17-OHCS after injection of zinc or ACTH, the maximum response has been noted after ACTH.





## BIOCHEMICAL RESULTS

*Fish (average values)*

Type of fish	Plasma 17-hydroxycorticosteroids in gamma per 100 cc
<i>L. rohita</i> (control)	16.2
<i>L. rohita</i> (zinc injected)	45.3
<i>L. rohita</i> (ACTH injected)	59.2
<i>C. mrigala</i> (control)	18.1
<i>C. mrigala</i> (zinc injected)	62.7
<i>C. mrigala</i> (ACTH injected)	78.3
<i>C. catla</i> (control)	22.6
<i>C. catla</i> (zinc injected)	65.3
<i>C. catla</i> (ACTH injected)	81.2

*Guinea pigs (average values)*

Type of animal	Plasma 17-hydroxycorticosteroids in gamma per 100 cc
Control guinea pigs	31.2
Zinc injected guinea pigs	93.2
Hypophysectomized guinea pigs	6.1
Hypophysectomized guinea pigs injected with zinc.	9.3

*Dogs (average values)*

Type of animal	Adrenal venous blood 17-OHCS output—gamma per minute	
	Before injection of zinc	After injection of zinc
Normal dogs	3.8	19.2
Ventral hypothalamectomized dogs with lesion of median eminence (within 24 hours).	1.6	1.9 (Pituitary infarcted in both the animals)
Dogs with lesion of hippocampus	12.3	39.4
Dogs with pituitary-island preparation	8.4	29.2
Hypophysectomized dogs (48 hours)	0	3.6



In guinea pigs, zinc leads to a rise in plasma 17-OHCS but there is no rise in the hypophysectomized animal.

In dogs there is a rise in adrenal venous blood 17-OHCS output after zinc injection. There is no rise in the ventral hypothalamectomized dogs with lesion of the median eminence and this can be accounted for the infarcted pituitaries in the animals. There is no rise in 17-OHCS output in hypophysectomized dogs. High basal level of 17-OHCS output is noted in dogs with lesion of the hippocampus and with pituitary-island preparation. The levels are further increased after injection of zinc. Diurnal fluctuation of 17-OHCS output is lost in dogs with lesion of the hippocampus.

### HISTOLOGICAL FINDINGS

*Fish.*—In *C. mrigala* the anterior interrenal tissue is found within the head kidney in close association with the cardinal veins. The cells of the anterior interrenal tissue are distributed as a single or double layer of cuboidal cells (Fig. 1). The second type of cell as described by Rasquin (1951) is also found. Sudanophilic substance is noted in the lumen of the blood vessels and only very faint reaction is noted in the cytoplasm. Demonstration of ascorbic acid granules was possible only in the blood vessels and not in the cytoplasm. Three hours after injection of zinc or ACTH, hypertrophy of the anterior interrenal cells was noted (Figs. 2 and 3). Cells were also found amidst the head kidney tissue (Fig. 4). Similar adrenal hypertrophy has been noted in *L. rohita* and *C. catla* after zinc or ACTH injection (Fig. 5). In *C. catla*, *C. mrigala* and *L. rohita* glomeruli are found in the head kidney. In *L. rohita* the anterior interrenal tissue is also found in the head kidney scattered among the renal components. At places such cells are many layered and are arranged in the fashion of a sheet with fibrous trabeculae surrounding groups of cells (Fig. 6). In the lumen of the cardinal veins the cells are protruded just like a pea in a pod (Fig. 7). The cells look vacuolated with haematoxylin stained nuclei. In *C. catla* some of the interrenal cells have got vesiculated nuclei with chromatins. Demonstration of sudanophilic substance and ascorbic acid granules in the vessels may be due to the release of the same from the adrenal cells.

The neurosecretory substance from the neurohypophysis is diminished (Fig. 8) and the basophils of the meso-adenohypophysis showed vacuolated appearance and the pituitary is congested after injection of zinc (Figs. 9, 10a, 10b). No alteration in basophils is noted at the third hour after ACTH injection.

Depletion of neurosecretory substance is noted in the cells of the preoptic nuclei and nucleus lateralis tuberis after injection of zinc (Figs. 11, 12, 13).

There was no change in the corpuscles of Stannius after injection of zinc or ACTH.

Close contact is present between the neurohypophysis and the adenohypophysis as from the neurohypophysis of fishes many fibre bundles pass



into the adenohypophysis similar to finger-like projections (Fig. 14). Neurosecretory substance may reach the pituitary cells either by direct contact or through the common capillary plexus of the neurohypophysis and adenohypophysis.

Pickford and Atz (1957) have proposed new set of terms for different parts of the pituitary gland :

- pro-adenohypophysis* for the anterior glandular region ;
- meso-adenohypophysis* for the middle glandular region or *ubergangsteil* ; and
- meta-adenohypophysis* for the pars intermedia or posterior glandular region.

Pickford (1958) states the nature and physiology of the pituitary hormones of fishes. Neurohypophysis produces oxytocic and vasopressor and antidiuretic hormones. Meta-adenohypophysis produces intermedin (MSH). In the meso-adenohypophysis growth hormone, corticotropin, thyrotropin and gonadotropins (LH and FSH) are produced. Prolactin, exophthalmos producing substance and erythropoietic factor (—ACTH) may also be produced in this lobe. The melanophore-concentrating hormone may be produced from the pro-adenohypophysis.

#### HISTOLOGICAL FINDINGS OF THE PITUITARY OF THE FISHES STUDIED IN THIS INVESTIGATION

*Pro-adenohypophysis*.—Acidophilic, basophilic and chromophobic cells are found in this lobe. Eosinophilic cells are plenty. The basophilic cells are very few in number. No marked change is noted in this lobe during a change in the sexual cycle.

#### MESO-ADENOHYPOPHYSIS OR THE ÜBERGANGSTEIL

Three cell types are present in this region of the pituitary but acidophilic and basophilic cells outnumber the non-granular chromophobic type. Large, deeply staining basophilic and acidophilic cells are found. Some of the basophilic cells contain vacuoles and specially in those fishes subjected to stress (Fig. 14a).

Seasonal change is markedly noted in this region of the pituitary. The nuclei of the basophil cells are large and there are nucleoli. There are spheres or globules in the cytoplasm of the basophils as noted by Scruggs (1951) (Fig. 15). The globules take acid stain. Such acidophilic globules may be discharged from the cells and lie amidst them (Fig. 16). These are transported to the target endocrine glands through blood vessels and are not the same as noted in the neurohypophysis and its branches. These are neurosecretory substances coming from the preoptic nuclei. These globular basophils increase in size and number and also the globules increase in size during the advent of the season with increased sexual activity. The rather indistinct outline of the acidophils becomes more distinct at the same time.



The fish pituitaries have been stained by Masson's trichrome stain, Gomori's aldehyde fuchsin stain and PAS stain technique. Meso-adenohypophysial cells are stained well by the above methods. The cells of the meta-adenohypophysis are stained pale pink with PAS stain. Thyrotropic and gonadotropic basophils could be differentiated in the pituitaries of the fishes studied here.

### META-ADENOHYPOPHYSIS

The cells in this portion of the pituitary gland may be granular or non-granular. In the granular variety there are acidophils and basophils. The basophils may be lightly or deeply stained. In the basophils vacuoles can be seen. Seasonal variation is not well marked in this lobe.

### NEUROHYPOPHYSIS

The neurohypophysis (Fig. 17) sends finger-like projections to all parts of the pituitary gland. Neuroglia cells are found more in the proximal part than distally. Three types of nuclei are found as observed by Scruggs (1951). Herring bodies have been also noted. There is seasonal variation in the colloid-like material. It is increased in the season with increased sexual activity. This material comes from the preoptic nuclei. Coloured fibres are noted in the neurohypophysis after Gomori stain. Gomori positive granules are also found along the fibres and are more numerous in the meta-adenohypophysis (Fig. 18). They are PAS negative.

### GUINEA PIGS

After injection of zinc there are changes in the adrenal cortex, pituitary and the hypothalamus. The adrenocortical cells show depletion of sudanophilic substance and loss of ascorbic acid granules. The cells of the zona fasciculata show cytolysis and vacuolation. The pituitary is congested and the basophil cells show degranulation and vacuolation. There is loss of neurosecretory substance from the neurohypophysis. The hypothalamus is congested and the supraoptic and paraventricular nuclei show loss of neurosecretory substance and vacuolation. In the hypophysectomized animals no marked change in the adrenal cortex has been noted due to the injection of zinc.

*Dogs.*—Histological changes are the same as noted in guinea pigs after injection of zinc. Adrenal cortex after ventral hypothalamectomy and lesion of median eminence has been studied previously (Roy, 1960a). After bilateral lesion of hippocampus, increased adrenocortical activity is noted whose degree is further increased by injection of zinc. The same is true for dogs with pituitary-island preparation.

### DISCUSSION

Our first knowledge about the anatomy and histology of the teleost adrenal cortex is from the pioneering work of Giacomini (from Chester



Jones, 1957). The adrenals were spoken of as 'anterior interrenal' and corpuscles of Stannius as 'posterior interrenal'. The anterior interrenal is situated in the head kidney. The head kidney has got lymphoid structure but in the *Fundulus* there are numerous glomeruli (Pickford, 1953*a, b*). The interrenal cells are situated around the cardinal veins and their tributaries. The literature about the morphology and histology of the adrenals of bony fish has been reviewed in many articles (Baecker, 1928; Callamand, 1943; Aboim, 1946; Rasquin, 1951; and Olivercau and Fromentin, 1954). In the salmonid interrenal (Spalding, *unpublished*—from Chester Jones, 1957) there were no lipid droplets and the Sudan stains colour the cytoplasm diffusely and faintly; cholesterol could not be demonstrated, the plasmas reaction was negative, there was no birefringent material; phospholipins were not shown after Baker's haematin method. Lipid droplets are absent from the interrenal cells and they do not react to many histochemical tests (Baecker, 1928; Rasquin, 1951).

In the teleost adrenocortical cells ascorbic acid could not be demonstrated histologically (Rasquin, 1951; Pickford, 1953*b*). In the present investigation sudanophilic substance or ascorbic acid granules could not be demonstrated in the fish adrenocortical cells. These were, however, demonstrated in the lumen of the blood vessels and this may be due to the release of the same from the adrenocortical cells. Hatey (1952) and Fontaine and Hatey (1954*b*) demonstrated the presence of ascorbic acid in the teleost adrenal by chemical method of Roe and Kuether (1943). In the eel, the ascorbic acid content of the interrenal is not altered after hypophysectomy (Hatey, 1954*a, b*). Mammalian ACTH injection could not change the ascorbic acid content of the hypophysectomized eel at 6°C. At 16°C mammalian ACTH leads to a 56 per cent. fall in the interrenal ascorbic acid content three hours after injection and the value returned to normal four hours after.

After hypophysectomy there was atrophy of the interrenal (Fontaine and Hatey, 1953). Olivercau and Fromentin (1954) found atrophy of the interrenal of the female eel one month after hypophysectomy. In the *Fundulus heteroclitus* L., the killifish, the interrenal of the hypophysectomized male did not show any degenerative change or any difference histologically from that of normal animals (Pickford, 1953*b*). After injections of fish pituitary preparations there was hypertrophy of the *Fundulus* adrenal cortex.

Hypertrophy of the interrenal and increased vascularization were noted in *Astyanax mexicanus* after implantation of carp pituitary, injection of mammalian ACTH, shock by cold treatment or to some extent after injection of Holtfreter's saline (Rasquin, 1951). Hypertrophy of interrenal cells has been noted in the present investigation in the types of fish as mentioned before after injection of zinc or ACTH.

The corpuscles of Stannius or the 'posterior interrenal' were thought to be the true interrenal of the teleost in the past. Vincent and Curtis (1927) thought that the 'Organ of Giacomini' rather than the corpuscles of Stannius was the teleost interrenal.



The corpuscles are small, irregularly spherical, white bodies situated either on the dorsal or ventral surfaces of the posterior part of the kidney. Usually a pair of bodies is found. Connective tissue surrounds the corpuscles and trabeculae proceed into them so that the cells are grouped into cords or lobules. Towards the centre of each group there is cell degeneration. The nuclei are small and basophilic and the cytoplasm is not markedly acidophilic (Fig. 19).

That the corpuscles of Stannius are not identical with the anterior interrenals can be understood from the following points :

(1) *Developmentally*.—The corpuscles are developed as bud-like evaginations from the wall of the pronephric duct (Garrett, 1942) and this mode is fundamentally different from that noted in the development of adrenocortical tissue which is developed from the proliferation of coelomic epithelial cells.

(2) *Histologically*.—The corpuscles are different from the anterior interrenals.

(3) *Pharmacologically*.—After injection of ACTH or cortisone the corpuscles of Stannius in *Astyanax* show no change whereas the interrenals show changes (Rasquin, 1951). In this present investigation there is no change in the corpuscles of Stannius after injections of zinc or ACTH whereas the same has been noted in the adrenocortical cells.

(4) *Experimentally*.—Pettit (1896) reported hypertrophy of the right corpuscle after removal of the left one in the eel, but normally there is wide variation in these corpuscles. Changes in these corpuscles were noted after injection of pilocarpine, curare and diphtheria toxin.

Vincent (1898) found that extirpation of the corpuscles in three eels had no effect on the animals.

The corpuscles of Stannius may be engaged in osmoregulation by production of osmoregulatory hormone/s or the hormones may have action on the reproductive system.

Callamand (1943) found that the extracts of corpuscles of the eel gave rise to melanophore reactions in *Cyprinus carpio* scales and contraction of the Rouget cells of capillaries.

Chester Jones (1957) states : 'On the grounds of absence of reaction to ACTH and to cortisone, and of their embryological origin, I think it unlikely that the wheel will turn full circle and adrenocortical function again be specifically assigned to these enigmatic bodies.'

(5) Ford (1959) found the corpuscles to be entirely lacking in hydrocortisone.

17, 21-dihydroxy 20-ketosteroids have been found in the plasma of salmon (Fontaine and Hatey, 1954a ; Fontaine, 1956). The compound is found in human plasma and its concentration indicates adrenocortical activity. High plasma 17-hydroxycorticosteroid level is found in the smolt stage (in process of migrating to the sea) of the salmon in comparison to that noted in the migrating parr. The increased 17-OHCS level is



due to the changing metabolism of the smolt and its preparation for a marine environment.

In the present investigation plasma 17-hydroxycorticosteroids in *C. mrigala*, *L. rohita* and *C. catla* have been studied. There is a rise in the levels after zinc or ACTH injection; the maximum response is noted after ACTH injection.

It can be stated therefore that the pituitary-adrenal axis operates in the types of fish studied here and it responds well to the stimulus of zinc or ACTH injection.

Changes noted in the basophil cells of the meso-adenohypophysis after zinc injection may be due to the liberation of ACTH from such cells. Neurosecretory changes observed in the preoptic and lateral tuberal nuclei after injection of zinc are evidences of increased activity. The types of fish examined here lack the hypophysiportal vessels but the neurohypophysis ramifies to a great extent in the pituitary and so thus the neurosecretion of the nuclei has got a very good chance to come to the adenohypophysial cells to activate them. This event occurs after injection of zinc or after any alteration of internal or external environment of the animal. Moreover meso-adenohypophysial secretion migrates to the meta-adenohypophysis.

Zinc may act on the caudal neurosecretory system causing discharge of the neurosecretion which activates the pituitary-adrenal axis. After injection of zinc there is loss of neurosecretory substance from the caudal neurosecretory system in the types of fish examined (Figs. 20, 21).

*Control of ACTH secretion (Fig. 22) :*

- (1) Peripheral blood level of corticoids ;
- (2) Nervous stimuli ;
- (3) Changes in external and internal environment and stress ;
- (4) Posterior pituitary hormones stimulating meso-adenohypophysial basophil cells ;
- (5) Discharge of ACTH which has migrated to the meta-adenohypophysis and neurohypophysis—in response to nervous stimuli ;
- (6) Substance P (Euler and Östlund, 1958) of the brain and spinal cord, histamine, zinc, potassium, etc., and changes in pH and chemistry of blood ;
- (7) Neurosecretion from preoptic and lateral tuberal nuclei activating the pituitary ;
- (8) Neurosecretory substance from the caudal neurosecretory system.

When zinc was administered intraperitoneally to guinea pigs, there was increased adrenocortical activity. There were histological changes and a rise in plasma 17-OHCS. Sobel *et al.* (1960) used zinc chloride and in this investigation zinc sulphate has been used. They found



increased corticoid excretion in guinea pigs after zinc chloride injection. Presence of the hypophysis is required for the increased adrenocortical response after injection of zinc as hypophysectomy abolishes the response after injection of zinc. The supraoptic and paraventricular nuclei show evidences of increased activity and the neurohypophysis shows loss of neurosecretion. After injection of zinc, neurosecretory substance is discharged from the supraoptic nuclei and this is carried through the hypophysiportal vessels to reach the anterior pituitary and to activate it to release more ACTH, which in turn stimulates the adrenal cortex.

There is increase in adrenal blood 17-OHCS output after injection of zinc in dogs. In two dogs with ventral hypothalamectomy and lesion of the median eminence no increase in 17-OHCS output was noted due to the infarction of the pituitaries. Pituitary is essential for the response after zinc injection as no appreciable effect was detected after zinc injection in hypophysectomized dogs. In dogs with bilateral hippocampal ablation and lesion of the fornices, high basal secretion of corticosteroids from the adrenals is noted. This indicates an inhibitory influence of this structure on the pituitary-adrenal axis. Zinc can augment the activity of the same axis in such lesioned dogs. Regarding the part played by archicortex, the first data demonstrating the role of the amygdaloid nuclei and the hippocampus in regulation of pituitary ACTH secretion have been furnished by Endrőczy, Lissák, Szép and Tigyi (1954) and Endrőczy, Martin and Bata (1958). Stimulation of the amygdaloid nucleus led to the activation of the stress mechanism and that of the hippocampus to its inhibition (Endrőczy, Lissák, Bohus and Kovács, 1959; and Lissák and Endrőczy 1960). The adrenocortical activity was considerably influenced by lesion of the septum affecting the afferent and efferent connections of the rhinencephalon. The archicortex has got a modifying and inhibitory influence over the behaviour of the animals and the activity of the pituitary-adrenocortical system (Endrőczy and Lissák, 1960). In the present investigation the same conclusion regarding the inhibitory control of the hippocampus and fornix system over the hypothalamo-pituitary-adrenal axis has been drawn *after experimental surgical lesion of the same system in dogs* as opposed to the stimulation experiments. After destruction of the hippocampus and fornix system the basal 17-OHCS output from the adrenal gland is increased which is further augmented after injection of zinc. Another important observation in such lesioned dogs is the loss of diurnal fluctuation of 17-OHCS output. Such disruption of the diurnal rhythm was noted in the monkeys with hippocampal ablation or fornix section by Mason (1957). Nauta (1960) states that 'the hypothalamus forms part of two neural circuits, one of which connects with the limbic forebrain structures, the other with a medial zone of the midbrain. . . . it seems possible to consider both circuits to be subdivisions of one and the same major circuit formed by largely multisynaptic neural connections connecting the limbic forebrain structures with a limbic midbrain area and vice versa, both limbs of the circuit relaying in large part in the hypothalamus. Viewed in this manner, the hypothalamus appears as a nodal point in a vast neural mechanism extending from the medial wall of the cerebral hemisphere caudalward to the lower boundary of the mesencephalon, and it seems logical to assume



that the functional state of the hypothalamus is continuously influenced by the prevailing activity patterns in the limbic forebrain-midbrain circuit as a whole.'

This limbic forebrain-midbrain circuit is important for the—

- (a) maintenance of the basal 17-OHCS output ;
- (b) diurnal fluctuation of 17-OHCS output ;
- (c) stress-induced increase in 17-OHCS output where the inhibitory influence is overcome by the stressing procedure (neural or humoral) ;
- (d) development of the 'stage of resistance' (Selye) after stress.

In dogs with pituitary-island preparation there is high basal level of 17-OHCS output. This is further increased after injection of zinc. Bard and Mountcastle (1948) state that the neurally isolated island of tissue (composed of ventral diencephalon, olfactory tubercles and hypophysis) thus left with adequate blood supply served to maintain a normal water and carbohydrate balance. In the present work with pituitary-island preparation no median eminence is present but still there is high basal level of 17-OHCS output. Therefore the ponto-cerebello-medullary complex leads to the discharge of neurosecretion in response to neural and humoral stimuli and then the neurosecretion circulating in the systemic blood stimulates the isolated pituitary to discharge more ACTH (Fig. 23). In such a preparation the inhibitory influence of the cortex and the hippocampus is lost and so the basal values are increased.

If we accept two centres, one hypothalamic and the other the ponto-cerebello-medullary complex, for the control of ACTH release then after hypothalamic lesion the stress should lead to rise in 17-OHCS output because such a stress as severe burn can break through the cerebral and hippocampal inhibition on the complex. This is more so as is evidenced by the ventral hypothalamectomy experiment and the influence of stress in such an animal (Roy, 1960a) but in this investigation both the dogs with ventral hypothalamectomy had infarcted pituitaries and so there was no response to the injection of zinc. In decorticated dogs, the inhibitory influence on the hypothalamus and the ponto-cerebello-medullary complex is not present and this can account for the high basal 17-OHCS output (Roy, 1960b unpublished). Obviously, then, response to stress should be more in such a preparation than in one animal with isolated pituitary, but such a situation is not found because of the presence of the hippocampus which has definitely one inhibitory influence on the hypothalamus.

*How zinc acts.*—Zinc is probably engaged in the enzyme systems of the neurosecretory cells and adenohypophysial cells.

### CONCLUSIONS

- (1) There is a rise in plasma 17-hydroxycorticosteroids after intraperitoneal injection of zinc sulphate at a dose of 0.1 millimoles or after intraperitoneal injection of ACTH at a dose of 0.2 I.U./g in the fish *L. rohita*, *C. mrigala* and *C. catla*.



(2) In guinea pigs there is a rise in plasma 17-hydroxycorticosteroids after intraperitoneal injection of zinc sulphate at a dose of  $3\mu\text{M}/100\text{ g}$  body weight. There is no rise in plasma 17-OHCS after injection of zinc in hypophysectomized guinea pigs.

(3) Adrenal venous blood 17-OHCS output is increased in dogs after intravenous injection of zinc sulphate at a dose of 0.2 millimoles. No rise is noted in ventral hypothalamectomized dogs with lesion of the median eminence and in hypophysectomized dogs.

(4) Dogs with lesion of the hippocampus or with pituitary-island preparation show high basal level of 17-OHCS output. Zinc injection leads to further increase.

(5) Diurnal fluctuation of 17-OHCS output is lost in dogs with lesion of hippocampus.

(6) Anterior interrenals and the pituitary are described in *C. mrigala*, *C. catla* and *L. rohita*. Very faint reaction is noted in the cytoplasm of the anterior interrenal cells after Sudan stain and demonstration of ascorbic acid granules was possible only in the blood vessels. Three hours after injection of zinc or ACTH there is hypertrophy of the anterior interrenal cells. The hypothalamus and the pituitary show increased activity.

(7) The hypothalamo-pituitary-adrenal axis of the guinea pig and dog shows increased activity histologically after injection of zinc. For zinc to act on the axis, the pituitary is to be present.

(8) Histologically it can be stated that the corpuscles of Stannius do not react to the injection of zinc or ACTH by increasing plasma 17-OHCS level as there is no change in the corpuscles. They may be engaged in osmoregulation by production of osmoregulatory hormone/s or the hormones may have action on the reproductive system.

(9) After injection of zinc in the fish, the neurosecretory substance from the preoptic and lateral tuberal nuclei is discharged to the adenohypophyseal cells wherefrom ACTH is liberated.

(10) Zinc may act on the caudal neurosecretory system of the fish and the neurosecretory substance thus discharged activates the pituitary-adrenal axis.

(11) The limbic forebrain-midbrain circuit (Nauta) is important for the—

- (a) maintenance of the basal 17-OHCS output ;
- (b) diurnal fluctuation of 17-OHCS output ;
- (c) stress-induced increase in 17-OHCS output where the inhibitory influence is overcome by the stressing procedure (neural or humoral) ;
- (d) development of the 'stage of resistance' (Selye) after stress.

(12) In pituitary-island preparation of the dog, the ponto-cerebello-medullary complex leads to the discharge of neurosecretion in response to neural or humoral stimuli which circulates in the systemic blood and stimulates the isolated pituitary to discharge more ACTH. In such a pre-





paration, as the inhibitory influence of the neocortex and the hippocampus is lost, the basal value of 17-OHCS output is increased.

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## Chapter 7

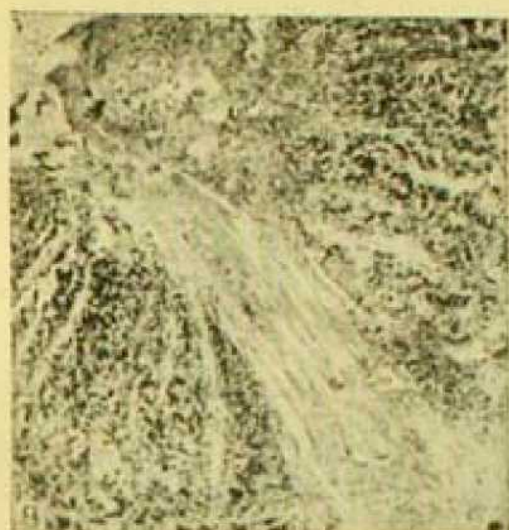


Fig. 8



Fig. 11



Fig. 14

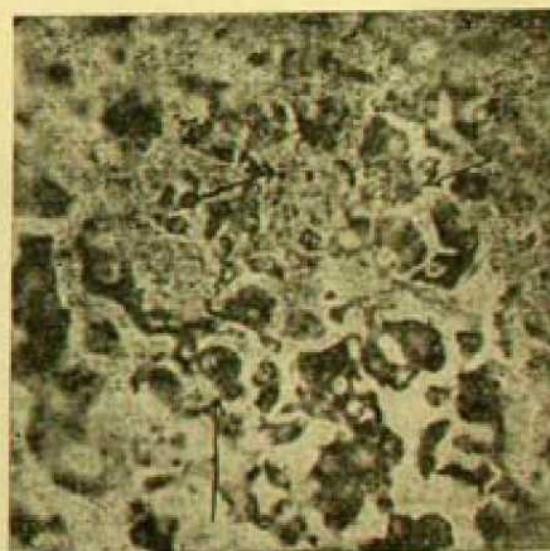


Fig. 15

- Fig. 8. The neurohypophysis and the adenohypophysial cells of *Labeo rohita* after injection of zinc. There is depletion of Gomori—positive substance from the neurohypophysis. On either side of the neurohypophysis meso-adenohypophysial cells are seen. (Gomori's CAHP stain.  $\times 50$ ).
- Fig. 11. Cells of the preoptic nucleus of *Cirrhina mrigala* showing Gomori-positive substance.  $\times 450$ .
- Fig. 14. Pituitary gland of *Labeo rohita* showing the different parts. (Haematoxylin and eosin stain.  $\times 50$ ).
- Fig. 15. Meso-adenohypophysial basophilic cells of *Cirrhina mrigala* showing globules—a seasonal change. (Gomori's aldehyde fuchsin stain.  $\times 450$ ).



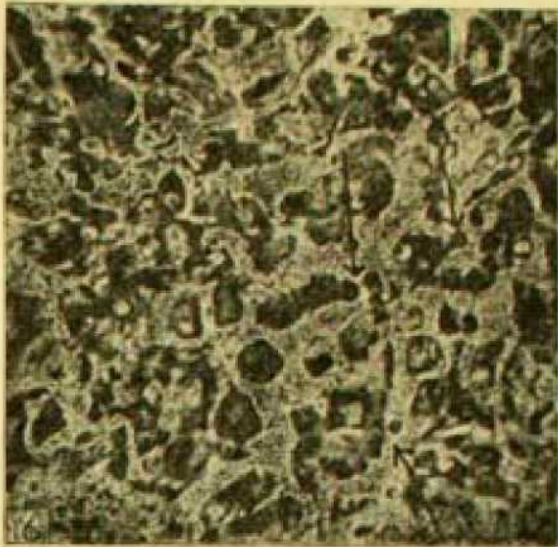


Fig. 16

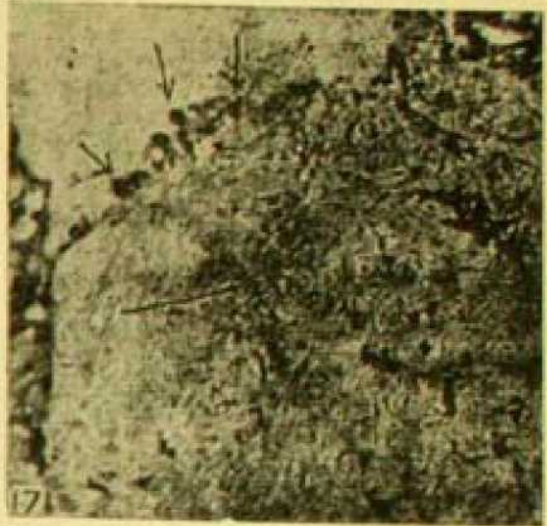


Fig. 17

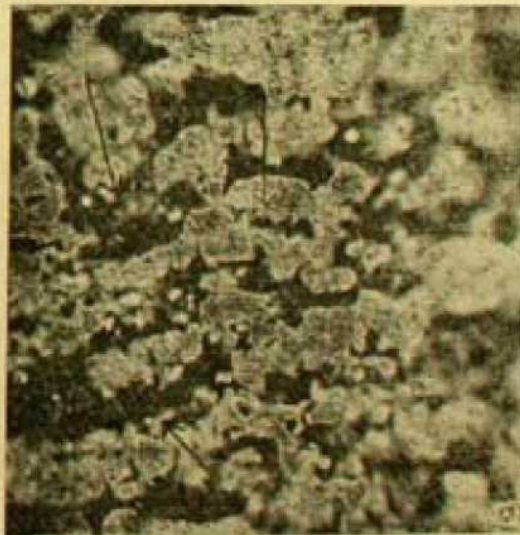


Fig. 18

- Fig. 16. The same section as in Fig. 15. The cells are filled with globules and some globules are noted amidst the cells. ( $\times 450$ ).
- Fig. 17. The neurohypophysis of *Cirrhina mrigala* showing Gomoriphillie colloid droplets. (Gomori's CAHP stain.  $\times 450$ ).
- Fig. 18. Meta-adenohypophysis of *Cirrhina mrigala* showing plenty of Gomori-positive substance among cell groups. (Gomori's CAHP stain.  $\times 450$ ).



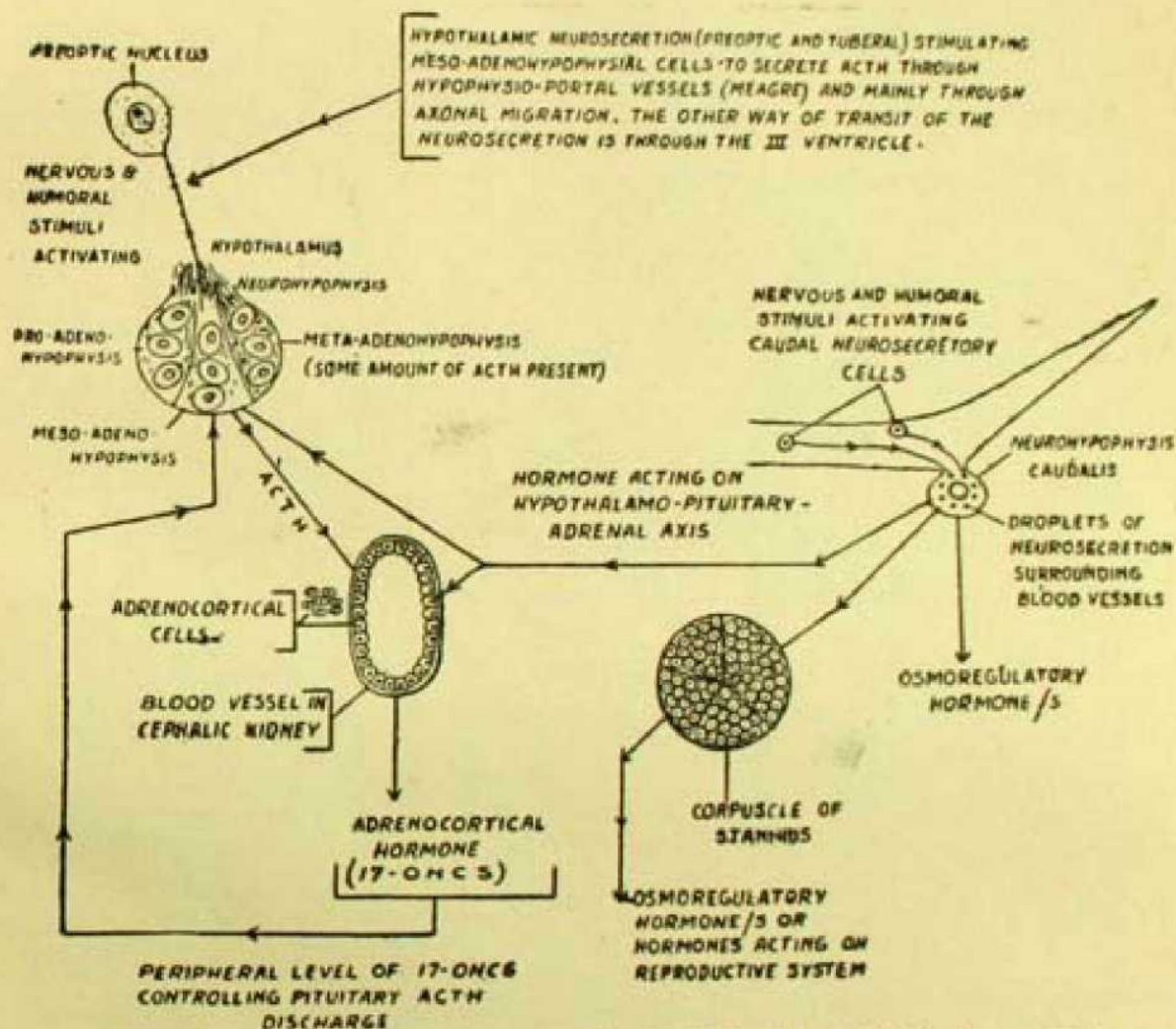
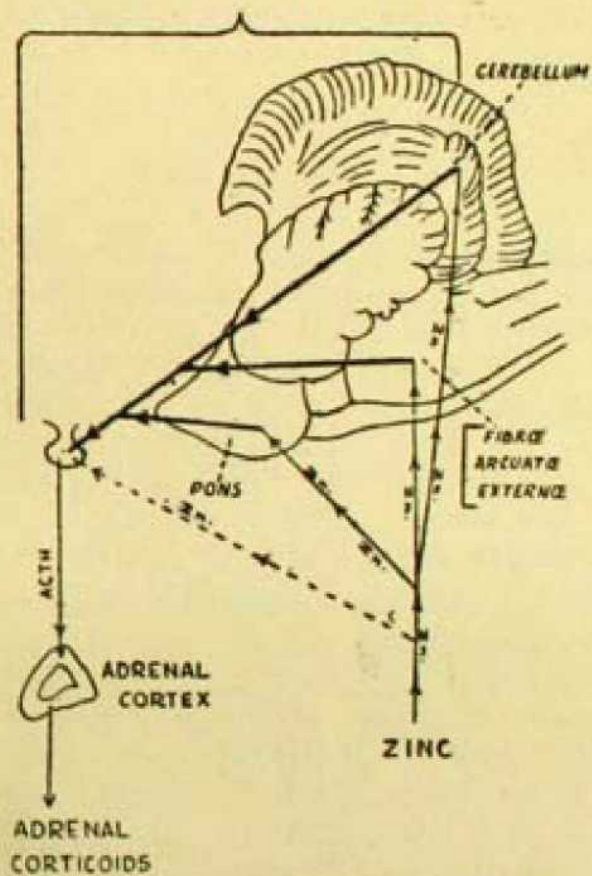


FIG. 22. Hypothalamo-pituitary-adrenal axis of the fish studied in the present investigation.



### NEUROSECRETION STIMULATING THE ANTERIOR PITUITARY.



**FIG. 23.** Mechanism of adrenal corticosteroid discharge in isolated pituitary experiment in dogs after injection of zinc.

Fig. 23



## CHAPTER—8

### PRODUCTION OF CORTICOSTEROIDS *IN VITRO* IN SOME INDIAN FISHES WITH EXPERIMENTAL, HISTOLOGICAL AND BIOCHEMICAL STUDIES OF ADRENAL CORTEX TOGETHER WITH GENERAL OBSERVATIONS ON GONADS AFTER HYPOPHYSECTOMY IN *O.* *PUNCTATUS*. (1964)

#### PART I

#### INTRODUCTION

There are different methods to study the biosynthesis of the adrenocortical hormones. (1) The adrenal gland can be perfused and it can form adrenocortical steroids from endogenous precursors (Hechter *et al.* 1951 ; Pincus *et al.* 1953 ; Vogt 1951). (2) Adrenal slices can produce corticosteroids when incubated in plasma or some other media (Haynes *et al.* 1953a, 1953b ; Saffran *et al.* 1951, 1952 ; Brady *et al.* 1953 ; Cooper *et al.* 1955). (3) Hechter (1952) points out that certain, but not other reactions proceed in homogenates. Corticosterone and compound F are formed from progesterone and 17-hydroxyprogesterone in homogenates.

Hypophysectomy abolishes the adrenocortical response after injections of formalin and insulin in rats. In the hypophysectomized pigeon however, these same stimuli lead to hypertrophy of the adrenal (Miller and Riddle 1942). Thus there is a species difference in the response. Vogt (1953) states that it takes about four times as long in the dog as it does in the rat for the same degree of adrenocortical atrophy to develop.

Pickford (1957) states that the effects of hypophysectomy develop rather slowly in fishes. Six weeks (at 20°C) must elapse before the target organs of the pituitary of *Fundulus* pass into a state of maximal regression and she states that experiments on hypophysectomized fish must not be made too soon after the operation.

Pickford and Vogt (1951) found that adrenocortical secretion is not abolished after hypophysectomy in dogs and this is due to the comparatively slow atrophy of the gland. Atrophy of the gland is only found in those animals which lived for twenty days or more. Adrenal cortex of the hypophysectomized dog becomes gradually more unresponsive to ACTH. Cortical hormone was found in the adrenal effluent of hypophysectomized dogs which were not maintained on ACTH and this study was carried out upto one month after hypophysectomy (Vogt 1953).

Roy (1960) studied about the influence of environment and stress on the adrenocortical response and found that in the hypophysectomized toads (*Bufo melanostictus*) 17-hydroxycorticosteroids could not be found



in blood under any of the experimental conditions (usual laboratory condition, constant illumination, kept in dark, fracture and scald). The plasma 17-OHCS were low in hypophysectomized guinea pigs. ACTH in such animals led to a rise in 17-OHCS content but it never approximated to the value noted in normal animals treated with ACTH. Totally hypophysectomized dogs (48 hours) showed almost zero values in adrenal venous 17-hydroxycorticosteroid output. With 50% hypophysectomy, increased response to the stress of fracture and burn and response to ACTH were noted. Ganong (1959) studied dogs six weeks after hypophysectomy. In these dogs the corticoid output was practically zero. Hume and Nelson (1955) found similar results.

Van der Vies *et al.* (1960) showed that the steroid formation rate *in vitro* and the plasma free corticosterone increase after formalin stress, environmental changes and injection of corticotrophin in rats. Hypophysectomy gave rise to a marked and rapid decline of adrenal activity *in vitro* and of the plasma free corticosterone. The hypophysectomized animals were sacrificed  $\frac{1}{2}$ , 1 $\frac{1}{2}$ , 2 $\frac{1}{2}$ , 4 and 24 hours postoperatively. After hypophysectomy there was rapid fall of the plasma corticosterone to almost zero; but the corticoid production *in vitro* did not reach such a low level. Van der Vies (1960) noted reduction of *in vitro* corticoid production after hypophysectomy in rats. Increased production was observed after exogenous histamine or corticotrophin. de Wied (1961) found that hypophysectomized rats did not show increased steroidogenesis *in vitro* after ether stress.

Hypertrophy of the anterior interrenal and increased vascularization were noted in *Astyanax mexicanus* after carp pituitary tissue implantation, mammalian ACTH injection, shock by cold treatment or to some extent after injection of Haltfreter's saline (Rasquin 1951). Atrophy of the anterior interrenal was noted after hypophysectomy (Fontaine and Hatey 1953). Olivereau and Fromentin (1954) noted atrophy of the interrenal of the female eel one month after hypophysectomy. Pickford (1953a) could not find any histological change in the anterior interrenal cells of *Fundulus heteroclitus* after hypophysectomy. However, there was hypertrophy of the anterior interrenal in both normal and hypophysectomized fish after injection of fish pituitary suspensions. Chavin (1956) studied hypophysectomized gold fish after one week and three weeks of the operation. After one week the adrenal cortex was normal or slightly below normal in extent and thickness. After three weeks the cortex was reduced in size and thickness though the number of cells apparently remained normal.

Cortisol and/or corticosterone are the main types of corticosteroids in fish (Fontaine and Hatey 1954; Phillips and Chester Jones 1957; Bondy *et al.* 1957; Hatey 1958; Phillips *et al.* 1959; Phillips, 1959; Chester Jones *et al.* 1959; Idler *et al.* 1959; Hane and Robertson 1959; Roy 1962).

Incubation experiments with head-kidney tissues of fishes have been done by Phillips and Mulrow (1959a) and Nandi and Bern (1960). In this present investigation the production of corticosteroids by the interrenal tissues of some Indian teleost fishes has been studied along with



some of the factors influencing steroidogenesis. Hypophysectomized fishes have also been included to note the histological changes in the anterior interrenal cells and the production of corticosteroids by them. Influence of ACTH, histamine and pitressin has also been noted.

### MATERIALS AND METHODS

The different types of mature fishes studied in this investigation are *Catla catla*, *Cirrhina mrigala*, *Labeo rohita*, and *Ophiocephalus punctatus*. The last variety has been included as these fishes are able to respire direct from the atmosphere and they are able to exist for prolonged periods out of their native elements due to accessory respiratory organs. Moreover they stand the experimental procedures well. All the varieties belong to the teleost group. They are all fresh water fishes and are of both sexes. The appearances of the head-kidneys are shown in figure number 1. In *O. punctatus* the disposition of the cardinal veins has been shown in figure number 2 after injecting radio opaque dye into the heart. Similar pattern is noted in other fishes. It is practically impossible to isolate the anterior interrenal cells from the adjacent vein and the kidney tissues and so exact quantitation of the steroids produced by incubation experiment is difficult. This problem can be solved to a certain extent if we dissect out the cardinal vein from the adjacent kidney tissues taking only bits of them along with the vein. This is done by sharp razors and fine pointed needles without lacerating the tissues much. Obviously some extramural adrenocortical cells were excluded from incubation. Tissues from both the head-kidneys have been taken. Usually ten fishes comprised one batch (125 to 200 mg. tissue). The tissues were divided into small pieces and incubated in 10 ml. conical flasks with 5 ml. of the incubating medium. Phillips and Mulrow (1959a) used mammalian incubation medium for fish tissues, but the medium after Nandi and Bern (1960) has been used by us. The stock solution was diluted with distilled water and made isosmotic to the blood of the fish which is approximately 0.16 M. In order to verify the importance of potassium as a stimulant to the adrenal cortex, the concentration of KCl in some experiments were doubled without altering the concentration of other ingredients and in some, increased concentrations of the media were used. The incubation was done from one and half hour to three hours at room temperature with or without oxygen. The media were changed every half an hour and they were pooled for final extraction. 0.1 and 0.2 units of Crooke's corticotrophin per 100 mg. of tissue were used in some experiments. Histamine (0.5 and 1.0 microgram per 100 mg. of tissue) and pitressin (0.05, 0.2 and 0.4 pressor units per 100 mg. of tissue) were also used in the incubation media.

In *O. punctatus* some other experiments were conducted after injecting distilled water (0.5 ml.), ACTH (2 I.U. per fish), histamine (5 microgram per fish) and pitressin (0.5 and 2 pressor units per fish) intraperitoneally. After three hours the tissues were collected for incubation experiment as mentioned before. The injection experiments were also done with hypophysectomized fishes (*O. punctatus*) in different weeks upto the fourth week. Corticotrophin (0.1 and 0.2 I.U. per 100 mg. of tissue), histamine



(0.5 and 1.0 microgram per 100 mg. of tissue), and pitressin (0.05, 0.2 and 0.4 pressor units per 100 mg. of tissue) were added in some incubation experiments with hypophysectomized *O. punctatus* (in different weeks after hypophysectomy). Some other animals got pretreatment with ACTH (0.1 I.U. daily for the whole period of the experiment). Hypophysectomy was done through the roof of the mouth. The mucous membrane was incised transversely and the osseous tissue in the midline was cut by means of a pair of fine pointed scissors. The osseous flap was elevated from its bed and the pituitary was removed by suction. Completeness of hypophysectomy was checked by serial section of the area after decalcification and staining the sections with haematoxylin and eosin. The batch containing the fish with incomplete removal of the pituitary was excluded from the series. Incubation experiments were done also with corpuscles of Stannius.

The pooled media and the tissues were extracted with two volumes of ethyl acetate thrice. The extract was washed once with 4 ml. of 0.05N NaOH, twice with redistilled water and evaporated *in vacuo* at 37°C. The period of contact with alkali was as brief as possible. The material was partitioned between hexane and water. Hexane fraction was discarded and the aqueous phase was extracted three times with two volumes of ethyl acetate. The extract was dried and dissolved in 0.1 ml. of methanol.

Whatman filter paper 20 was cut into lanes and the methanolic final extract was spotted. Similar runs were given with different standards at room temperature. The solvent systems used were cyclohexane : benzene : methanol : water (4 : 2 : 4 : 1) (Phillips and Mulrow 1959a) for 24 hours and toluene : methanol : water (4 : 3 : 1) (Bush 1953) for 6 to 8 hours. The steroids were identified by noting the mobility with reference to standards, mobility of the acetylated compounds, iodine reaction, with triphenyltetrazolium chloride, NaOH fluorescence, Silber-Porter reaction, ultraviolet absorption of ethanol eluates over the range of 220 to 300m  $\mu$  in a spectrophotometer and the sulphuric acid spectrum.

The head-kidney tissues were fixed in 10% formaldehyde and paraffin sections stained by Masson's trichrome stain and haematoxylin and eosin stain.

## RESULTS

**Histology :** The anterior interrenal cells of *L. rohita*, *C. catla* and *C. mrigala* have already been described by Roy (1962a). In these fishes the cells are located within the head-kidney in close association with the cardinal veins. The anterior interrenal tissue consists of a single or double layer of cuboidal cells. Cells in groups were also found scattered in the head kidney tissue. In *O. punctatus* similar pattern was noted (Fig. No. 3). After injection of ACTH, histamine and higher doses of pitressin there was hypertrophy of the anterior interrenal cells and the layers of cells were multiple. After injection of distilled water intraperitoneally, increased activity was not noted. The anterior interrenal cells were examined in different weeks following hypophysectomy. By the end of first week, practically there was no change in the cells (Fig. No. 4). Fourteen



days after, there was reduction in the size of the cells without alteration in their number. Some of the anterior interrenal cells were vacuolated. The vacuolations were more frequently met with, when the specimens were examined by the end of the third and the fourth week (Fig. Nos. 5 & 6). In these weeks the cell size further diminished when we can speak that true adrenocortical atrophy has occurred after hypophysectomy. In *O. punctatus* that starts in the second week, intensified in the third week and is complete by the end of the fourth week. Hypophysectomized fishes supplemented by daily dose of ACTH show well maintained anterior interrenal cells, in different weeks. Hypertrophy of the cells with increased thickness and number of the layers were noted. The ACTH treatment was stopped and the animal was sacrificed next day. No change was, however, noted in the chromaffin type cells.

A single dose of ACTH by the end of the first and second week after hypophysectomy led to hypertrophy of the anterior interrenal cells. Similar changes were noted after injections of histamine and higher doses of pitressin (Fig. Nos. 7 & 8). Marked change was not histologically demonstrable by the end of the third and fourth week after hypophysectomy when the above drugs were injected.

#### *Steroid production in vitro :—*

Oxygen is an important requirement in the incubation medium for the production of steroids. In its absence steroid production was much hindered. With the increase of the period of incubation (three hours) more steroid productions were noted but there was no production of any new steroid. By increasing the concentration of the incubating media and by increasing the potassium concentration only, increased steroid productions were noted without production of any new steroid. Incubation of corpuscles of *Stannius* did not produce cortisol and corticosterone.

In all the types of fishes cortisol and corticosterone could be identified. Cortisol was indentified by noting the mobility with reference to the standard run on a parallel lane. The substance gave a strong reaction with sodium hydroxide. Silber-Porter reaction was positive with a peak at 410 m $\mu$ . Ethanolic eluates gave a maximum absorption at 240 m $\mu$  and the sulphuric acid spectrum gave a typical peak reaction at 290 m $\mu$ . Reaction with triphenyltetrazolium chloride was positive. Corticosterone was identified by noting the mobility with reference to the standard run on a parallel lane. It gave strong reaction with sodium hydroxide and the reaction with triphenyltetrazolium chloride was positive. Ethanolic eluates gave maximum absorption between 238 m $\mu$  and 242 m $\mu$ . Silber-Porter reaction showed maximum absorption at 350 m $\mu$ . This is characteristic for corticosterone as shown by Silber and Porter (1954). Sulphuric acid spectrum showed maximum absorption at 285 m $\mu$  and subsequent peaks were at 330, 373 and 455 m $\mu$ . This is characteristic for standard corticosterone as has been stated by Zaffaroni (1953) and this has also been observed in our laboratory. The acetylated compound ran parallel with the standard acetate.



A zone was detected apart from cortisol and corticosterone when incubation experiment was carried with excess amount of tissue and this reacted positively with NaOH and TPTZ, and gave maximum absorption at 240 m $\mu$  with ethanolic eluates. The Silber-Porter reaction was negative. The substance ran parallel with standard aldosterone. Possibly this compound is aldosterone.

With ACTH and pitressin (.2U) production of cortisol and corticosterone appeared to be more. When doses were doubled, there was augmented production of the same. With histamine there were increased productions in some experiments but not in all. With pitressin (0.05 U) there was no increase in the steroid production.

By the end of the first week after hypophysectomy, production of cortisol and corticosterone was normal in comparison to that in the non-hypophysectomized fishes. The steroid production was comparatively small by the end of the second week after hypophysectomy. By the end of the third and fourth week production further diminished but still the atrophied cells could produce detectable amounts of steroids. No new steroid appeared during the period of studies with the hypophysectomised fishes.

ACTH, piteressin (0.2U) and histamine led to increased production of cortisol and corticosterone when added to the incubation media containing the anterior interrenal tissues from hypophysectomized fishes after first and second week. The production further increased when the doses were doubled. With pitressin (0.05U) there was no increase in corticoid production. Such an increased production, however, was not noted in the third and fourth week after hypophysectomy even when the doses were doubled.

Increased production of cortisol and corticosterone was noted in the incubation experiments in all the weeks after hypophysectomy when the animals received daily doses of ACTH. No new steroid bands appeared. The production was more in comparison to that noted in the experiments with the nonhypophysectomized animals.

Single doses of ACTH, histamine, and pitressin (2U) three hours before the *in vitro* experiment in the first and second week after hypophysectomy led to increased production of cortisol and corticosterone. By the end of the third and fourth week the drugs could not increase the production of the steroids to a great extent.

#### *Amount of steroid production :*

The method for taking the anterior interrenal tissues for incubation experiments has been described. Here the weight of the cardinal vein and very little amount of the head-kidney tissue have been included in the total weight of the tissues for incubation. The quantitation was done by noting the absorption of the compounds in ultraviolet at 240 m $\mu$  (ethanolic). It was also done by comparing with known amounts of standard steroids treated with sodium hydroxide and triphenyltetrazolium chloride



The values of steroid production after injection of distilled water, and single doses of ACTH, histamine and pitressin (2U), three hours before the *in vitro* experiment in the second week after hypophysectomy have been presented here. The production of cortisol was 8.0 microgram per gram of tissue per hour in the distilled water injected animals. With the injection of ACTH, histamine and pitressin, the production of cortisol was 15.0, 10.5 and 18.5 microgram per gram of tissue per hour respectively. In the distilled water injected animals the production of corticosterone was 2.0 microgram per gram of tissue per hour. After the injection of ACTH, histamine and pitressin the production of corticosterone was 3.8, 2.6 and 4.2 microgram per gram of tissue per hour respectively.

### DISCUSSION

In the present investigation with fishes there was hypertrophy of the anterior interrenal cells after injection of ACTH, histamine and pitressin. True adrenocortical atrophy started in the third week and was complete by the fourth week after hypophysectomy. Increased vacuolation of the anterior interrenal cells is an important finding in the third and the fourth week after hypophysectomy. When the hypophysectomized fishes get daily doses of ACTH, the anterior interrenal cells are well maintained. In the first and second week after hypophysectomy, ACTH, histamine and pitressin (higher doses) can stimulate the anterior interrenal cells whereas no such change is noted in the third and the fourth week. The adrenocortical cells are unresponsive. With lower doses of pitressin there was no change.

Oxygen, period of incubation, concentration of the media and concentration of potassium are modifying agents for the production of steroids by the anterior interrenal cells incubated *in vitro*. Potassium is an important stimulus for the adrenocortical secretion of mammals (Vogt 1951; Roy 1953). In the fishes also, potassium stimulates steroidogenesis. Incubation of corpuscles of Stannius did not produce cortisol and corticosterone. Fontaine and Leloup-Hatey (1959) could extract detectable amounts of corticosteroids from the corpuscles of Stannius in salmon. Phillips and Mulrow (1959b) found a failure of corpuscles of Stannius from winter flounder (*Pseudopleuronectes americanus*) to synthesize adrenocorticosteroids *in vitro*. Ford (1959) could not find corticosteroids in extracts of the corpuscles from carp and salmon. In this present investigation also such a failure was encountered. This may be due to a species difference or due to small quantity of the tissues taken for incubation experiment (same amount as the anterior interrenal tissues). Whereas the anterior interrenals could produce the steroids, the corpuscles did not produce it.

The incubation of the anterior interrenal cells produced cortisol and corticosterone. Production of cortisol was more in comparison to that of corticosterone. Possibility of the presence of aldosterone was also there.

ACTH and pitressin (at higher doses) could augment the production of steroids in incubation experiments. Zaffaroni (1952) incubated adrenal slices with ACTH. He did not get a net increase in the total amount of



corticosteroid production. Hechter (1952) gives the explanation that corticosteroid synthesized in adrenal slices is balanced by destruction and so there is no net increase. However, Cooper *et al.* (1955) and others got an increase in the production of corticosteroid after ACTH using mammalian adrenal slices. In this present investigation increase in corticosteroid production has been achieved after ACTH using nonmammalian tissues. It is to be decided also whether the preparations *in vitro* of the anterior interrenal cells of fishes can produce the steroids or we get simple washing out of the already formed steroids in the tissues. That the cells actively produce the steroids is proved by the increase of steroid production by ACTH and pitressin. Pitressin can stimulate the anterior interrenal cells at higher doses. The steroidogenic action of vasopressin has been demonstrated by Hilton *et al.* (1959) in the isolated perfused adrenal of the dog. Vasopressin increases the adrenal venous blood 17-OHCS concentration in hypophysectomized dogs (Hume and Nelson 1957). Royce and Sayers (1958) showed that adrenal ascorbic acid is depleted in the hypophysectomized rat after vasopressin. Sayers (1960) summarized the action of vasopressin as (1) it acts directly on the adrenal cortex (2) it inhibits degradation of ACTH when added to anterior pituitary homogenates (3) it releases ACTH from extrapituitary binding sites and (4) it releases ACTH from the adenohypophysis.

Increased production of the steroids with histamine in the hypophysectomized fishes was not found in all the experiments. Production of cortisol and corticosterone was good in the first and second week after hypophysectomy. In the third and fourth week the atrophied anterior interrenal cells could produce the steroids but not to the extent as noted in the first and second week. In the first and second week after hypophysectomy ACTH, pitressin (higher doses) and histamine increased the production of steroids. The atrophied anterior interrenal cells in the third and fourth week could not increase the production to a great extent. Pretreatment with daily dose of ACTH after hypophysectomy led to good amount of steroid production. Increased production of cortisol and corticosterone was noted in the first and second week after hypophysectomy when single dose of ACTH, histamine and pitressin (2U) was injected three hours before the *in vitro* experiment. This was not observed in the third and fourth week after hypophysectomy. Pitressin (0.5U) failed to show any change in the first and second week after hypophysectomy. Quantitation of the steroids produced in the *in vitro* experiment with the fish anterior interrenal cells is possible. The production of cortisol was 8.0 microgram per gram of tissue per hour in the distilled water injected animals in the second week after hypophysectomy. With the injection of ACTH, histamine and pitressin (2U) there was increased production of cortisol. In man the production of cortisol is 20.8 microgram/gram/hour (Cooper *et al.* 1955); in guinea pigs it is 13 microgram/gram/hour (Roy 1962).

### CONCLUSION

Steroid production *in vitro* has been studied with the anterior interrenal tissues of the teleost fishes (*Catla catla*, *Cirrhina mrigala*, *Labeo*



*rohita*, and *Ophiocephalus punctatus*). Cortisol and corticosterone could be identified in these fishes. Production of aldosterone could also be identified.

Oxygen is required for the production of steroids. Period of incubation has got an influence over the quantitative production of the steroids. When the period of incubation was increased more steroids were produced. Similarly increased concentration of the incubation media and increased potassium concentration stimulated the production of the steroids more.

The corpuscles of Stannius did not produce cortisol and corticosterone. *In vitro* production of cortisol and corticosterone was increased with ACTH and pitressin (at higher doses). Histamine could increase the production in some experiments but not in all.

Steroid production by the anterior interrenal cells of the hypophysectomized fishes was normal in the first week, started to diminish in the second week and the production further diminished in the third and the fourth week. Response to ACTH, pitressin (higher doses) and histamine was good in the first and second week but this was not observed in the third and fourth week after hypophysectomy whether the drugs were injected intraperitoneally or added to the incubation medium. With lower doses of pitressin there was no increase in the response. Hypophysectomized animals having daily doses of ACTH showed no difference with the controls having the same treatment regarding the production of corticosteroids *in vitro*.

The production of cortisol and corticosterone in the second week after hypophysectomy after injection of distilled water was 8.0 mcg./Gm./hour and 2.0 mcg./Gm./hour respectively. After injection of ACTH, histamine and pitressin (higher doses) the production of cortisol and corticosterone was 15.0, 10.5, and 18.5 mcg./Gm./hour and 3.8, 2.6, and 4.2 mcg./Gm./hour respectively.

Histologically hypertrophy of the anterior interrenal cells with increase in the layers of cells were noted after injection of ACTH, histamine and pitressin (higher dose). With lower dose of pitressin there was no change. No change was noted in the cells in the first week after hypophysectomy. Two weeks later the cell size reduced, some cells showing vacuolation. True atrophy occurred in the third and the fourth week and the cells showed vacuolation. Hypophysectomized fishes having daily doses of ACTH showed well maintained anterior interrenal cells. Hypertrophy of the cells was noted.

## PART II

In continuation of the previous work the following are further details including histological, biochemical and experimental observations. *Anaemia*—The fishes (*O. punctatus*) were studied in different weeks after hypophysectomy upto six weeks. Anaemia started in the third week and gradually intensified upto six weeks.



Dhar (1948) found the haemoglobin content of the blood of *O. punctatus* to be 8 gms. per 100 ml. This was done by Haldane-Gower's standard haemoglobinometer.

Pickford (1959a) found the hypophysectomized male killifish to be frequently extremely anaemic as could be judged from the pallor of the gills. All the fishes were somewhat anaemic and a few were severely affected. When growth hormone was administered upto two months, there was no significant effect (Pickford, 1953b, 1954a). Certain preparations of thyrotropin might change the condition (Pickford, 1954b). At present it cannot be assessed whether the anaemia after hypophysectomy is either due to the faulty metabolism or due to the lack of a specific erythropoietic factor. But the lack of hypophysis in the fish definitely gives rise to anaemia as is seen in mammals. Slicher (1961) found anaemia in hypophysectomized *F. heteroclitus* (Linn.).

*Changes in the weight and length:* Various factors modify the growth of the hypophysectomized fish. They are (a) regression of gonads, (b) enlargement of liver, (c) oedema due to renal lithiasis, (d) diet, (e) environmental change, (f) seasonal change and other factors. The length of the operated fishes (*O. punctatus*) did not show any change whereas in the control group under the same environmental condition and diet an increase in length was observed. Regarding weight no difference was noted in the operated fishes as compared to the unoperated within six weeks after hypophysectomy. After this period there is a definite loss of weight whereas the controls began to grow normally. Pickford (1953a) observed an average increase in weight of the same order as that in the controls during the first two months, but subsequently the operated fish lost weight except a few that developed oedema.

*Kidney:* In none of the experimental fishes, the head kidneys were enlarged. Pickford (1953a) found enlargement of the head-kidneys in the *Fundulus* due to obstruction of the urinary duct. In *O. punctatus* the head-kidneys were atrophic in all animals after the second week of hypophysectomy. Urinary lithiasis was not observed in any of the fishes. The atrophy of the head-kidneys may be partially due to atrophy of the anterior interrenal elements after hypophysectomy.

*Osmoregulation after hypophysectomy:* *O. punctatus* could survive for a considerable period after hypophysectomy in fresh water. Chavin (1956) found that *Carassius* was able to survive either in fresh water or in 0.7% saline after hypophysectomy. Pickford (1953a) found that after complete hypophysectomy *Fundulus* could not survive in fresh or diluted sea water.

*Gonads (Testis):* Pickford (1953a) studied the hypophysectomized male killifish. The spermatogonial region of the cortex remained unaffected. Spermatocytes and nests of spermatids were visible in few cases. The tubules were empty and there were no spermatozoa. Matthews (1939) and Burger (1941) studied the effects of hypophysectomy on the gonads of *Fundulus*.

*Interstitial cells of Leydig:* Some workers think that the development and sexual maturity of the fish are due to the presence of male hor-



none which is liberated by the Leydig cells. Others could not find these cells in sexually mature fish. Marshall and Lofts (1956) found two types of arrangement of the Leydig cells in the teleost fishes :

- (a) Typical vertebrate arrangement of endocrine interstitial Leydig cells—found in *Gasterosteus aculeatus*, *Tilapia spp.*, *Clupea sprattus*, *Latimeria*, *Seylliorhinus* and *Chimaera*.
- (b) The Leydig cell homologue in the lobule walls of certain teleost fishes—these are named as 'lobule boundary cells'—found in *Esox lucius*, *Salvelinus willughbii*, *Labeo (sp. ?)* and *Salmo salar*. The crypt wall in the teleost testis is composed essentially of fibroblasts. In the pike and char these become seasonally modified into lipoidal, cholesterol-positive gland-cells similar to the interstitial cells of higher vertebrates. Annual interstitial cell cycle also occurs in these lobule boundary cells. In most vertebrates there is a gonadotrophic influence on the intertubular connective tissue. The perilobular connective tissue is similarly influenced.

In the catfish (*Heteropneustes fossilis*) Ghosh and Kar (1952) found the seminiferous tubules to be closely packed together and there was very little interstitial space. Interstitial elements could however, be found at certain places. These consisted of capillaries, typical fibroblast cells and round cells with a spherical nucleus. The latter appeared to be secretory in nature and the authors thought that they might be the Leydig cells.

Champy (1923a, b) and van Oordt (1923, 1924, 1925) stated that the interstitial cells in *Tinca vulgaris*, *Phoxinus laevis*, *Gasterosteus pungitius* L., and *Xiphophorus helleri* do not produce male hormone. Courrier (1921b) and Weisel (1949) failed to find interstitial cells in primitive elasmobranchs and in two teleost species. Courrier (1921b, 1922c) and Kolmer and Scheminzky (1922) noted the interstitial cells in other species. Changes in the interstitial cells occur just before and during the breeding season but this may be partly obscured by the distension of the testis with sperm (Courrier, 1921a, c, 1922a, b; Craig-Bennett, 1931; Marshall and Lofts, 1956). Ghosh and Kar (1952) did not observe any cyclic change in the Leydig cells of the catfish. Thus there is production of androgenic hormone throughout the year in this species.

*Leydig cells in O. punctatus* : The interstitial cellular elements are of two different types. Fibroblasts and true interstitial cells are found (Fig. No. 9). These interstitial cells have got the same characteristics as those of higher vertebrates. There is no seasonal cyclic change in the Leydig cells of *O. punctatus*.

*Testis of O. punctatus* : The testes were collected in different months of the year and studied macroscopically and histologically.

*Microscopic examination* :—Spermatogenesis is found throughout the whole year and the seminiferous tubules are packed with spermatozoa.



No seasonal variation is noted in the activity of the testis. The basement membrane of the seminiferous tubule is formed of fibrous tissue. Different spermatogenetic stages are found e.g. spermatogonia, spermatocytes, spermatids and spermatozoa. The spermatogonia are the more prominent with round shape and spherical nucleus (Fig. No. 9).

*Ovary of O. punctatus* :—The ovaries are two elongated structures one on each side of the body. During the breeding season they are enlarged, hyperaemic and occupy a greater part of the body cavity. There is maximum weight of the ovaries during this season. Multiple yellowish ripe ova could be seen through the thin covering. These changes were observed during the months of April to July. During the months of August to November the ovaries gradually decrease in size and become less vascularized until in the month of November they become infantile and no ovum could be seen. During the months of December to March the ovaries gradually increase in size with increase in weight and vascularity.

*Corpus luteum in the ovary of O. punctatus* :—In *O. punctatus* true corpus luteum-like structure is not found. However, some atretic follicles may be found. Failure to form mature ovum may lead to hypertrophy of the follicle and thus a preovulation corpus luteum is found. This has been observed in teleosts by Wallace (1903), Bretschneider and Duyvenè de Wit (1947) and Stolk (1951). Hoar (1957) states that the use of the term "corpus luteum" may be questioned. Bretschneider and Duyvenè de Wit (1947) found that in the reptiles true post-ovulation corpora lutea appeared first phylogenetically. Atz (1957) said "we have seen that there is no indisputable evidence for the endocrine nature of the so-called corpus luteum of the teleost and that the only definite evidence for elasmobranchs is negative."

*Effect of hypophysectomy on gonads of O. punctatus* :—In the male fishes there was loss of weight of the gonads and the maturation of the spermatogonia was inhibited after eight weeks of hypophysectomy. There were no spermatozoa. The interstitial cells of the testis seem to be atrophied after hypophysectomy. In the dog there is reduction in the size of the Leydig cells with increased fat content after hypophysectomy (Huggins and Russel 1946). There is marked involution of the Leydig cells in other mammals after hypophysectomy. Testicular anterior pituitary grafting in the mice after hypophysectomy leads to repair of the atrophic tubules and the involuted Leydig cells (May 1955).

Oliverreau (1954) noted the presence of activity in the ovary for about four months after hypophysectomy in *Anguilla anguilla*. Vivien (1939) observed atrophy of ovaries with fewer oocytes and inhibition of their growth in two to three months after hypophysectomy during spawning season in *Gobius paganellis*. Vivien (1941) studied the activities of the gonads in *Gobius paganellus* in different periods of the year after the operation. The activities differed with maximum change just before spawning and during winter gametogenesis. There was no change in the gonads which were in immature condition. In *O. punctatus* there was atrophy



of the ovaries after hypophysectomy. Maximum change was noted during the heightened activity of the gonads.

*Pituitary stalk section in O. punctatus* :—This operation is distinctly advantageous in discerning the influence of the hypothalamus on the pituitary, whether it is through the hypophyseo-portal vessels or through a nervous mechanism. It is well known that definite pituitary portal vessels do not exist in fishes but the plexus of vessels between the neurohypophysis and the adenohypophysis may take up similar function of the portal vessels (Green 1951). Vivien (1941) noted retardation of oviposition in two hypophysectomized female *Gobius* with successful pituitary grafts in the anterior chamber of the eye.

In this present investigation the pituitary stalk region of *O. punctatus* was exposed and the pituitary stalk was divided by a fine needle. After severance of the stalk the pituitary was left in place and the wound was closed. In another set of experiment the pituitary was grafted into the anterior chamber of the eye after hypophysectomy (autogenous graft). After pituitary stalk section the vascular and nervous connexion with the hypothalamus was disrupted and there was atrophy of the neural lobe component. The anterior lobe was subsequently well vascularized. The gonads in male and female fishes did not show any deviation from normal in whichever part of the year this operation was performed. In fishes with successful grafting of the pituitary in the anterior chamber of the eye, there was atrophy of the gonads. During the period of observation (upto six weeks) no marked atrophy of the anterior interrenal cells was noted (Fig. No. 10). Thus it seems that the connexion with the hypothalamus is important for the integration of the pituitary-gonad-axis and some chemical substance is definitely required for the purpose—it may be from the tuberal nuclei. The autografted anterior pituitary can maintain the anterior interrenal cells.

#### *Lesion of the hypothalamus and gonadal activity in O. punctatus* :—

Schönherr (1955) stated that there was a marked reduction in all reproductive and parental activities in one male stickle-back having extensive hypothalamic lesion. In the present investigation surgical lesions of the hypothalamic region have been attempted and the gonads of the fishes have been examined. When I started this experiment the mortality rate in the immediate postoperative period was very high; subsequently it was brought down to 50%. Such fishes showed atrophy of the gonads within a month after the operation.

#### *Lesion of the forebrain and gonadal activity in O. punctatus* :—

Forebrain lesions on *O. punctatus* have been done after drilling the skull and suction of the desired brain area by a thin needle. Sometimes surgical removal by a thin needle was also done. All the experiments were terminated within a month.

- (a) In unilateral lesions no change in the pituitary-gonadal axis was seen.



- (b) In bilateral lesions gonadal atrophy was a marked feature both in the males and the females.
- (c) In incomplete lesions no change was noted.

Noble (1939) stated "complete removal of the forebrain of fish has a detrimental effect upon the pituitary with the result that the gonads degenerate. If small rudiment of the forebrain be left, these fish may be brought to spawning by pituitary replacement therapy. However, all social patterns such as those of synchronization during spawning and breeding are lost in those fish which have suffered such an extensive destruction of the striatum." Pflugfelder (1954) found that the reproduction in *Lebistes* was not prevented after total ablation of the forebrain. Some changes were noted by Aronson (1948) and Kamrin and Aronson (1954) after forebrain lesion. Aronson (1957) states "From the above studies it is evident that the forebrain does not function as the organizer of sexual and parental behavior patterns; rather, it seems to act as a general energizer or facilitator of centers and mechanisms lower in the brain."

There was atrophy of the anterior interrenal cells in *O. punctatus* after bilateral ablations of the forebrains. In the early part of the experiment and in incomplete lesions hypertrophy of the anterior interrenal cells was noted (Fig. No. 11).

#### *Nucleus tuberis lateralis* :—

Histological studies of this nuclear group, other neurosecretory cell groups and pituitary have been conducted after staining the sections by Masson's trichrome method, haematoxylin and eosin, fuchsin-paraldehyde method and chromealumhaematoxylin-phloxine method of Gomori. The fixation fluid for the tissues were Helly and Bouin.

Kappers, Huber and Crosby (1936) state that the nucleus tuberis lateralis is to be regarded as merely a ventrocaudal extension of the nucleus pre-opticus magnocellularis. The nucleus consists of small polygonal cells and it is situated at the base of the hypothalamus. The area has got visceral functions. In the teleosts the tuber cinereum contains four groups of nuclei. Large cells comprise the nuclei. Charlton (1932) called them as the nucleus ventralis tuberis with anterior, posterior, intermediate and lateral parts. The lateral part is known as the nucleus tuberis lateralis. In certain fishes e.g. *Melanocetus murrayi*, *Esox lucius* and *Pseudopleuronectes americanus* there was subdivision of the nucleus tuberis lateralis into a pars medialis and a pars lateralis. In *O. punctatus* these divisions are not met with. Stahl (1957) found the two divisions of the tuberal nuclei in *Mugil capito*. In *Morone labrax* the pars medialis is absent. The cells of the nucleus tuberis lateralis are large, polygonal in shape and situated in groups. The more anteriorly situated cells are bigger than those situated posteriorly. The nuclei present variegated appearance in *O. punctatus*. Some are small and some big. During the advent of the breeding season characteristic changes occur in the cells. They attain bigger size and the intranuclear inclusions (Fig. No. 12) are well found.



Multilobed nuclei are not found in this type of fish. The nucleoli are oval and appear to be reddish. During the hyperactive state multiple nucleoli are found. The intranuclear inclusion bodies take up acidophilic stain and may be of different shapes—irregular, oval or rhomboid. Some nuclei are crescentic in appearance, the concave surface is bordered by basophilic cytoplasm. We meet with intranuclear neurosecretion in *O. punctatus*. Smaller neurosecretory masses coalesce to form bigger masses and they are destined for the cytoplasm. This pronounced feature is noted during the breeding season. The means by which the neurosecretory material comes to the cytoplasm have been cited by Palay (1943). Seasonal variation in these cells has been described by Palay (1943) and Stahl (1957). During the breeding season the big cells in *O. punctatus* are filled with neurosecretory material (Gomori negative) in the cytoplasm amidst the Nissl bodies. Cells with different sizes of vacuoles are met with. The bigger vacuoles are formed by the coalescence of smaller ones. The vacuoles contain neurosecretory material. At some places *hydroencephalocriny* is met with. Other way of transit of this neurosecretion is via the axonal paths to the pituitary. Vascular pathway forms the third way of transit as it is found that these are in close connection with blood vessels and intravascular neurosecretion has been detected in *O. punctatus*. Stahl (1953) thought about a correlation between the activity of the tuberal nuclei and the development of the gonads. Adenohypophysial control was also there. Certain basophil cells of the anterior lobe produce a gonadotrophic hormone which is responsible for sexual maturation. These basophil cells are under the control of the tuberal nuclei. The gonadotrophs in *O. punctatus* in the mesoadenohypophysis are increased in number during the advent of the breeding season and they show hyperactivity with the maturation of the gonads. Thus in this fish it is found that increased activity of the mesoadenohypophysial basophil cells with vacuolations, and increased gonadal activity (Fig. No. 13) come together. The link is, exteroceptive impulses → tuberal nuclei → gonadotrophs → gonads. Though, in the fish true hypophyseal-portal vessels are not present still the vessels in the ramifications of the neurohypophysis may take up the function of them. And thus the neurosecretion may activate the pituitary. Neurosecretions from the third ventricle may be absorbed by vessels and reach the mesoadenohypophysis. Regarding the maturation of the gonads the role of caudal neurosecretory system is important. Its action is through the mesoadenohypophysial basophil cells. The hyperactivity of the hypothalamo-pituitary-gonadal axis during the breeding season is met with in *C. mrigala*, *C. catla* and *L. rohita* (Fig. Nos. 14, 15, 16).

#### *Effect of hypophysectomy on caudal neurosecretory system :*

The caudal neurosecretory system in *O. punctatus* has got a cell station, axonal paths and a storage-release-center i.e. the urohypophysis. Normally, in the urohypophysis, isolated collection of neurosecretory material in the form of colloid is very scanty (Fig. No. 17), but these collections are abundant after hypophysectomy (Fig. No. 18). Possibly this is due to the absence of the target organ on which this caudal neurosecre-



tion acts. *O. punctatus* having autogenous hypophysial grafts in the anterior chamber of the eye does not show the abundance of the neurosecretory collections in the urohypophysis.

#### *Hypophysectomy and praeoptic cells :*

Palay (1953) noted the effects of hypophysectomy on the praeopticohypophysial pathways in *Fundulus heteroclitus*. He examined two male specimens 15 weeks after hypophysectomy. In both these brains there was marked deficiency of neurones. The large cells disappeared and only a few medium sized cells remained (10% to 20%).

In the present investigation the praeoptic cells were examined upto two months after hypophysectomy. Many of the praeoptic cells were absent (Fig. No. 19). Those surviving had neurosecretory materials in the form of granules and conglomeration of granules to bigger droplets. Surviving cells had axons which showed loaded appearance due to the transit of the neurosecretory material. At the proximal stump there was accumulation of neurosecretory material produced by the surviving cells (Fig. No. 20).

#### *Transection experiment of the Praeopticohypophysial tract :*

Kobayashi *et al.* (1959) did this experiment on young marine gobies, *Lepidogobius lepidus*. They found prompt accumulation of aldehyde—fuchsin staining bodies at the proximal cut ends of the axons. Two weeks after the operation there was partial or complete degranulation of the neurohypophysis. There was no change in the adenohypophysis.

The technique of the operation in *O. punctatus* is same as for hypophysectomy except the removal of the pituitary. When the pituitary is exposed, the area in between it and the optic chiasma is cut transversely by the tip of a fine needle. Some of the animals died of shock and haemorrhage. Those surviving were sacrificed at weekly intervals upto the third week.

There was accumulation of the neurosecretory material in the area proximal to the cut. The distal part of the severed axons were devoid of it. The depletion of the neurosecretory material in the neurohypophysis depended on the totality of section of the tract. In very few animals such was the case and in these the neurohypophysis was devoid of the neurosecretory material. Those with incomplete division showed some material in the neurohypophysis. In incomplete section majority of the praeoptic cells were unchanged. In near-total section the reverse was true. The adenohypophysis in general was not much altered ; but many of the mesoadenohypophysial basophils showed degranulation and vacuolated appearance. It may be due to the stimulation of the operation leading to increased ACTH discharge as, such animals had changes in the anterior interrenal cells showing hyperactivity.

#### SUMMARY

Hypophysectomized *O. punctatus* manifested anaemia and there was no increase in length and weight. There was atrophy of the head-kidneys.



These animals could survive in fresh water for a considerable period after hypophysectomy. Leydig cells are found in *O. punctatus* and there is no seasonal cyclic change in those cells. There is no seasonal variation in the activity of the testis. Different spermatogenetic stages are noted e.g. spermatogonia, spermatocytes, spermatids and spermatozoa. Seasonal variation is noted in the ovaries. There is apparently no true corpus luteum-like structure. In male fishes the weight of the gonads diminished after hypophysectomy and there was inhibition of the maturation of spermatogonia after eight weeks. There were no spermatozoa. Atrophy of the interstitial cells of the testis also occurred. Ovaries atrophied after hypophysectomy. Pituitary stalk section leads to disruption of the vascular and nervous connection with the hypothalamus. The neural lobe atrophied but the anterior lobe was well vascularized. There was no change in the gonads. Hypophysectomized fishes with autografted pituitary in the anterior chamber of the eye had atrophy of the gonads but anterior interrenal cells did not show atrophy. There was gonadal atrophy in *O. punctatus* after lesion of the hypothalamus. In unilateral forebrain lesions there was no change in the pituitary—gonadal axis. Gonadal atrophy was noted after bilateral lesions. There was no change after incomplete lesions. Bilateral lesions of the forebrains led to atrophy of the anterior interrenal cells. With incomplete lesions hypertrophy was noted. In *O. punctatus* the nucleus tuberis lateralis is not divided into pars medialis and pars lateralis. Increased activity is noted in these cells during the advent of the breeding season. The tuberal neurosecretion stimulates the mesoadenohypophyseal gonadotrophs. Caudal neurosecretory system has got an important role in the maturation of the gonads via the pituitary. During the breeding season hyperactivity of the hypothalamopituitary-gonadal-axis is also noted in *C. mrigala*, *C. catla* and *L. rohita*. After hypophysectomy there is accumulation of neurosecretory material in the urohypophysis. Many of the praeoptic cells are absent after hypophysectomy. The surviving cells were active in elaborating the neurosecretory material. At the proximal stump there was accumulation of the material. Similar findings were noted after division of the praeopticohypophysial tract. In incomplete lesion there was some neurosecretory material in the neurohypophysis and there was accumulation of the same proximal to the section. In near-total lesion the neurohypophysis was devoid of neurosecretory material. Mesoadenohypophysial basophils showed increased activity.

### PART III

#### BIOCHEMICAL OBSERVATIONS

##### *Materials and Methods :*

In the following experiments both male and female *O. punctatus* (about six inches in length) have been used. In the control group 0.5 ml. distilled water was injected intraperitoneally per fish. The experiment with forced swimming was continued for one hour. The effects of ACTH, pitressin, protopituitrin and histamine were studied at doses of 2 I.U./fish,



2U/fish, 0.2U/fish and 5 mcg./fish respectively. These drugs were injected intraperitoneally. Blood was collected after severing the tail in a heparinized pot three hours after the injections.

Caudal neurosecretory and diencephalic extracts have been prepared from *O. punctatus* and *L. rohita* as described by Roy (1962b) and injected intraperitoneally into *O. punctatus* in both normal and hypophysectomized (1 week) fishes at a dose of 2 mcg./fish. Three hours after the injections the blood was collected as described before.

The effect of surgery was also noted in *O. punctatus* by exposing pituitary for hypophysectomy but without removing the pituitary and this involved injury to both soft and skeletal tissues at the roof of the mouth. Blood was collected 1h, 3h, 5h, 12h and 24 hours after the operation.

Hypophysectomy was done in *O. punctatus* as described before. The animals were studied in the 1st week, 2nd week, 3rd week and 4th week after hypophysectomy. The effect of ACTH, pitressin and histamine was noted in all these weeks after hypophysectomy. The drugs were administered intraperitoneally. In the first and second week, pitressin was used at two dose levels. Animals with intraperitoneal injection of 0.5 ml. distilled water/fish served as controls. Blood was collected as described before three hours after the injections.

The collected blood samples were immediately centrifuged, the plasma separated and plasma 17-hydroxycorticosteroids were estimated after the method of Silber and Porter (1954).

## RESULTS

In the control group of fishes the plasma 17-hydroxycorticosteroid was 18.0 mcg./100 ml. There was practically no difference in the plasma steroidal value between the distilled water injected group and the control normal fishes. Therefore the distilled water injected group has been taken as control group here. Forced swimming for one hour increased the value to 53.6. ACTH, pitressin, protopituitrin and histamine augmented the activity of the pituitary-adrenal-axis (Table No. I). Increased

TABLE I  
Plasma 17-OHCS (mcg. per 100 ml. in  
*O. punctatus* under different experimental  
conditions).

A	B	C	D	E	F	G
1	21.0	55.0	45.6	67.0	62.0	26.4
2	18.0	49.2	50.2	60.5	55.9	31.7
3	17.6	53.4	54.1	63.9	60.1	35.0
4	19.0	50.1	57.0	59.0	56.7	29.4
5	21.5	57.3	49.8	62.4	54.9	32.0
6	14.4	60.1	43.6	68.1	60.7	30.1
7	17.0	51.9	47.0	65.3	57.8	29.7
8	17.2	52.0		63.3	53.7	
9	15.1				61.0	
10	19.2					
Average	18.0	53.6	49.6	63.7	58.1	30.6

A=No. of experiment. B=Control 5 ml D.W./fish. C=Forced swimming for one hour. D=ACTH 2U/fish. E= Pitressin 2U/fish. F=Protopituitrin 0.2U/fish. G=Histamine 5 mcg./fish.



plasma 17-OHCS levels were noted after the injection of diencephalic and caudal neurosecretory extracts in nonhypophysectomized *O. punctatus* (Table No. II). The extracts were taken either from *O. punctatus* or from *L. rohita*. There was no increase in the response when the extracts were injected into hypophysectomized *O. punctatus*. This proves that pituitary is essential for the mediation of the response.

TABLE II

Plasma 17-OHCS (mcg. per 100 ml.) in *O. punctatus* after injection of neurosecretory extracts.

A	Caudal neurosecretory extract from same group of fishes 2 mcg./fish		Diencephalic extract from same group of fishes 2 mcg./fish		Caudal neurosecretory extract from <i>L. rohita</i> 2 mcg./fish		Diencephalic extract from <i>L. rohita</i> 2 mcg./fish	
	B	C	D	E	F	G	H	I
1	49.0	18.7	46.2	17.6	50.5	21.1	51.4	20.2
2	42.2	16.5	40.9	15.1	44.1	18.8	45.0	21.5
3	51.6	20.0	48.0	16.3	45.9	19.0	41.8	17.3
4	44.2	17.0	41.6	19.0	49.0	16.5	44.2	19.7
5	48.1	18.9	47.0	15.0	42.0	17.0	47.0	18.9
Average	47.0	18.2	44.7	16.6	46.3	18.5	45.9	19.5

A=No. of experiment. B=Non-hypophysectomized. C=1st week after hypophysectomy. D=Non-hypophysectomized. E=1st week after hypophysectomy. F=Non-hypophysectomized. G=1st week after hypophysectomy. H=Non-hypophysectomized. I=1st week after hypophysectomy.

The plasma 17-OHCS level increases after surgery and the maximum peak is reached at third hour. The value comes down to normal at about twentyfour hours after surgery (Table No. III).

TABLE III

Plasma 17-OHCS (mcg. per 100 ml.) in *O. punctatus* at different hours after surgery.

SURGERY					
A	B	C	D	E	F
1	34.2	46.4	36.4	26.4	16.9
2	27.6	49.4	38.2	29.0	18.4
3	29.4	42.7	31.4	36.1	25.5
4	38.1	53.9	40.9	31.8	23.1
5	24.0	51.3	37.2	27.9	21.9
6	29.0	56.2	32.4	25.4	19.6
7	36.1	44.1	39.1	30.0	20.4
8	32.7	48.2	34.2	32.8	22.0
Average	31.4	49.2	36.2	29.9	20.9

A=No. B=1 hour. C=3 hours. D=5hours. E=12 hours. F=24 hours.

In the first and second week after hypophysectomy, the plasma 17-OHCS levels were 16.2 mcg./100 ml. and 15.7 mcg./100 ml. respectively in the control group (Table No. IV). These values were low in the third and the fourth week. ACTH had a good stimulating effect on the an-



TABLE IV  
Plasma 17-OHCS (mcg. per 100 ml.) in hypophysectomized *O. punctatus* after injections of ACTH, histamine and pitressin.

	Pitressin										Pitressin									
	1st week after hypophysectomy					2nd week after hypophysectomy					3rd week after hypophysectomy					4th week after hypophysectomy				
A	B	C	D	E	F	G	H	I	J	K	L	M	N	O	P	Q	R	S		
1	..	19.1	46.7	17.6	67.0	29.0	14.7	41.2	45.9	65.1	26.2	11.6	20.1	21.4	17.1	6.1	3.4	5.4	4.1	
2	..	16.2	50.5	20.0	63.5	27.6	16.5	36.9	46.7	59.5	23.1	13.5	18.4	23.2	15.2	5.2	3.5	4.9	2.9	
3	..	15.4	42.3	19.1	69.1	26.2	18.9	39.7	19.4	62.8	28.9	12.0	21.1	17.5	20.3	3.6	4.2	6.5	3.7	
4	..	16.6	46.9	22.4	60.5	28.4	15.6	43.2	17.1	58.4	24.2	15.2	17.9	20.7	15.4	4.7	4.6	5.6	3.3	
5	..	14.5	45.1	16.1	61.7	25.0	14.3	42.0	16.4	57.3	25.0	14.1	19.1	22.1	14.5	5.9	4.3	5.1	2.6	
6	..	17.9	40.4	17.0	70.0	28.7	17.7	44.1	17.5	60.2	22.7	15.8	17.5	17.9	14.7	6.2	6.0	5.0	2.7	
7	..	14.0	49.3	15.1	66.4	30.1	15.0	36.0	14.2	56.5	27.1	13.0	16.6	18.0	16.1	4.4	3.9	4.0	2.8	
8	..	16.3	44.0	21.6	64.1	26.9	14.9	40.2	15.0	61.0	23.8	15.9	16.2	20.0	17.2	4.0	4.0	5.2	3.0	
Average		16.2	45.6	18.6	65.3	27.7	15.7	40.4	16.5	60.1	25.1	13.9	18.3	20.1	16.3	5.0	4.1	5.2	3.1	

A = No. of Experiment. B = 0.5 ml D.W./fish. C = ACTH-2 I.U./fish. D = 0.5 P.U./fish. E = 2 P.U./fish. F = Histamine 5 mcg./fish. G = 0.5 ml. D.W./fish. H = ACTH 2 I.U./fish. I = 0.5 P.U./fish. J = 2 P.U./fish. K = Histamine 5 mcg./fish. L = 0.5 ml. D.W./fish. M = ACTH 2 I.U./fish. N = 2 P.U./fish. O = Histamine 5 mcg./fish. P = 0.5 ml. D.W./fish. Q = ACTH 2 P.U./fish. R = 2 P.U./fish. (Pitressin). S = Histamine 5 mcg./fish.



terior interrenal cells in the first and second week. However, this was not so in the third and the fourth week. With higher dose of pitressin good response was noted in the first two weeks. The response was not present in the fourth week. With lower dose level there was no increased response in the first two weeks after hypophysectomy. Increased response was noted in the first two weeks after injection of histamine. In the last two weeks there was no response.

### DISCUSSION

Stress leads to increased activity of the pituitary-adrenal-axis in mammals. This is also found in the present investigation in teleost fish (*O. punctatus*). Plasma 17-OHCS rises after forced swimming for one hour, after injections of ACTH, pitressin, protopituitrin and histamine. Increased response is also noted after surgery, the maximum response is at the third hour and steroidal value comes down to normal, twenty hour hours after surgery. Diencephalic and caudal neurosecretory extracts were prepared as described by Roy (1962b). These had stimulating action on the pituitary-adrenal axis of fish. The presence of the pituitary is essential for the mediation of the response in the hypophysectomized fishes after the injection of the extracts. The effect of hypophysectomy on the anterior interrenal cells of *O. punctatus* develops very slowly. In the first two weeks after hypophysectomy good amount of plasma 17-OHCS was found proving thereby that in these weeks the anterior interrenal cells could produce the steroids even when the pituitary was absent. In the third and the fourth week the production was less ; but still the atrophied cells could produce some amount of steroids though at a much lower level. Small dose of pitressin required the presence of the pituitary for its action on the anterior interrenal cells, i.e., it acts first on the anterior pituitary to liberate ACTH and which in turn stimulates the anterior interrenal cells. This is proved in the hypophysectomized fishes as there is no increased response in steroidal production with small dose of pitressin and it does not act directly on the anterior interrenal cells at a smaller dose. At a higher dose pitressin acts directly on the anterior interrenal cells because increased plasma 17-OHCS is noted in hypophysectomized fishes. In the later weeks after hypophysectomy, the anterior interrenal cells become insensitive to ACTH, pitressin and histamine. These drugs had no stimulating effect on the anterior interrenal cells in the 4th week after hypophysectomy.

Forced swimming and injections of ACTH, pitressin, protopituitrin and histamine lead to increased activity of the pituitary-adrenal-axis in *O. punctatus*. Surgical measures also stimulate the same axis, the maximum response being noted at the third hour after such procedure. There is increased activity of the axis after injections of diencephalic or caudal neurosecretory extracts. The pituitary is essential for this response as there is no increase in plasma 17-OHCS after these injections in hypophysectomized fishes.

Hypophysectomy leads to lowering of the plasma 17-OHCS level and this is pronounced in the fourth week. Pitressin in large dose has got



a direct stimulating action on the anterior interrenal cells; with smaller dose the presence of the pituitary is essential for the response. After hypophysectomy the anterior interrenal cells become gradually insensitive to the actions of ACTH, pitressin and histamine.

### SUMMARY

Production of cortisol, corticosterone and aldosterone could be detected in *in vitro* incubation experiments with the anterior interrenal tissues of the teleost fishes (*C. catla*, *C. mrigala*, *L. rohita* and *O. punctatus*). Oxygen is a definite requirement for the production of steroids. Period of incubation, concentration of the medium, and concentration of potassium are modifying factors in corticosteroid production. The corpuscles of Stannius did not produce cortisol and corticosterone. Increase in the production of cortisol and corticosterone was noted after ACTH, pitressin (higher doses), and histamine. Histologically and biochemically it can be stated that the true adrenocortical atrophy occurs in the third and the fourth week after hypophysectomy. Response to ACTH, pitressin and histamine is variable in the hypophysectomized fishes and there is no stimulation in the later period after hypophysectomy. Hypophysectomized animals with daily doses of ACTH behave normally. In the second week after hypophysectomy the production of cortisol and corticosterone was 8.0 mcg./Gm./hour and 2.0 mcg./Gm./hour respectively.

In hypophysectomized *O. punctatus* there was anaemia with no increase in length and weight and atrophy of head-kidneys. There is no seasonal cyclic change in Leydig cells and in the activity of the testis. Ovaries manifest seasonal variation. Hypophysectomized male fishes showed atrophy of gonads and there was inhibition of maturation of spermatogonia. Atrophy of interstitial cells and ovaries was noted. Pituitary stalk section did not give rise to any change in the gonads. Hypophysectomized fishes with autografted pituitary in the anterior chamber of the eye showed atrophy of gonads but there was no atrophy of the anterior interrenal cells. Gonadal atrophy was noted after lesion of hypothalamus and forebrain. There was atrophy of the anterior interrenal cells after bilateral lesion of forebrains. Increased activity of the nucleus tuberis lateralis is noted during the breeding season. Mesoadenohypophyseal gonadotrophs are stimulated by tuberal neurosecretion. The importance of caudal neurosecretory system in maturation of the gonads through the action of the pituitary is also there. The same features are also noted in *C. mrigala*, *C. catla* and *L. rohita*. Accumulation of neurosecretory material is noted in the urohypophysis after hypophysectomy. Many of the praeoptic cells are absent after hypophysectomy. In near-total lesion of the praeopticohypophyseal tract the neurohypophysis was devoid of neurosecretory material.

Forced swimming, ACTH, pitressin, histamine and surgical measures lead to increased activity of the pituitary-adrenal-axis in *O. punctatus*. Diencephalic or caudal neurosecretory extracts have also got the stimulating action. Pituitary is essential for this response. Low plasma 17-OHCS is noted in the fourth week after hypophysectomy. Pitressin in





low dose requires the presence of the pituitary for its stimulating action. Higher doses have got a direct action on the anterior interrenal cells. The anterior interrenal cells become gradually insensitive to the actions of ACTH, pitressin and histamine after hypophysectomy.

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## Chapter 8

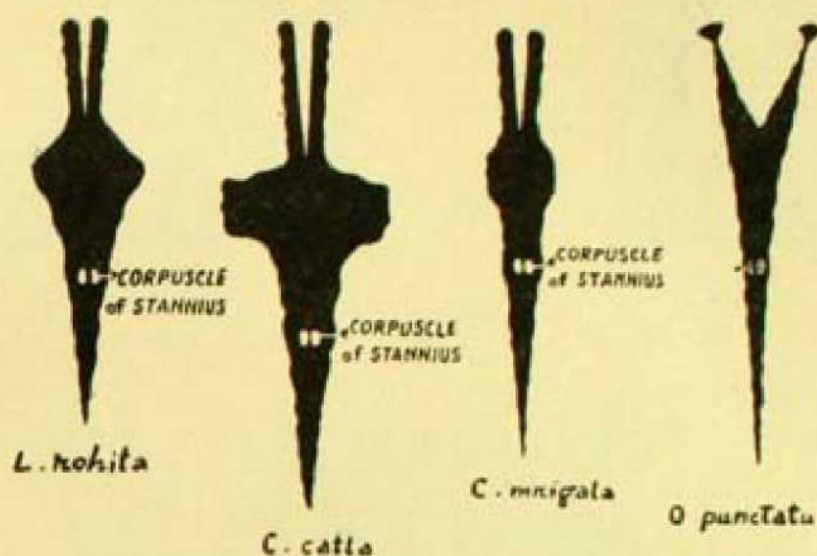


Fig.1. Appearance of head kidneys

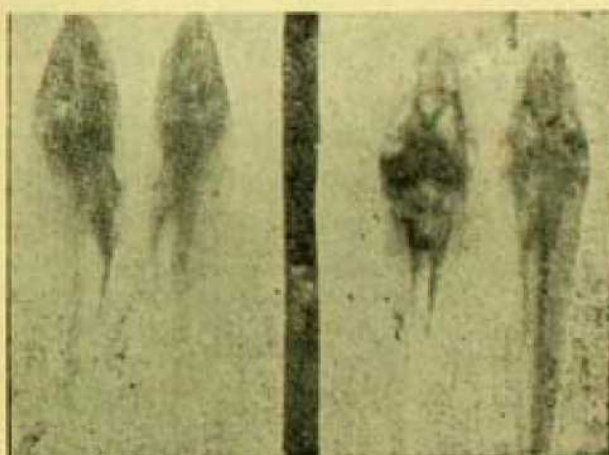


Fig. 2



Fig. 13

- Fig. 2. Radiograph showing disposition of cardinal veins after injection of radio-opaque dye into the heart.
- Fig. 13. Ovary of mature *O. punctatus* during the advent of breeding season corresponding with increased activity in the tubular cells. Haematoxylin and eosin stain.  $\times 50$



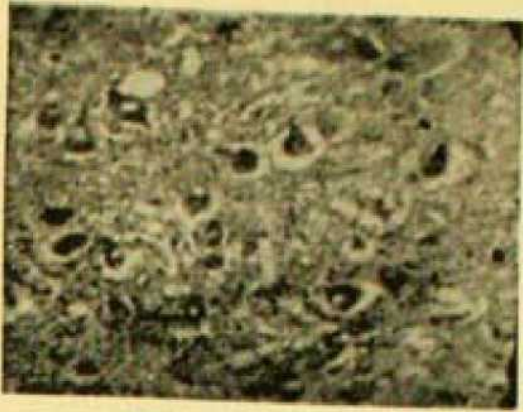


Fig. 14

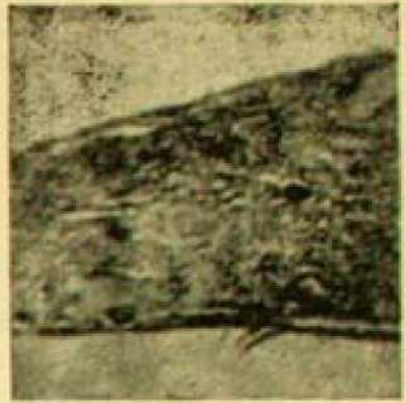


Fig. 18



Fig. 17



Fig. 15



Fig. 20

- Fig. 14. Tuberal cells of *O. mrigala* showing heightened activity in the tuberal cells during breeding season. Masson's trichrome stain.  $\times 215$ .
- Fig. 15. Tuberal cells of *O. catla* showing heightened activity in the tuberal cells during breeding season. Gomori's CAHP stain.  $\times 215$ .
- Fig. 17. Normal urohypophysis of *O. punctatus*. Gomori's CAHP stain.  $\times 215$ .
- Fig. 18. Urohypophysis of hypophysectomized *O. punctatus* in the second week showing accumulations of neurosecretory material in the form of colloid. Gomori's CAHP stain.  $\times 450$ .
- Fig. 20. Appearance of the stalk region of the pituitary in *O. punctatus* after hypophysectomy (third week) showing accumulation of neurosecretory droplets. Gomori's CAHP stain.  $\times 50$ .



## CHAPTER 9

### HYPOPHYSIO-PORTAL CIRCULATION, HYPOTHALAMUS, PITUITARY AND ADRENAL OF THE *BUFO MELANOSTICTUS* AND CHANGES IN THE HYPOTHALAMUS, PITUITARY AND ADRENAL AFTER FRACTURE AND OTHER STRESSES

(1957)

It has been agreed in recent years that the hypothalamus-pituitary-adrenal axis plays a great role in homeostasis in different species. In the present paper the changes in the axis have been studied in *Bufo melanostictus* after application of different types of stresses.

#### MATERIALS AND METHODS OF THE WORK

Types of stress :—

- (a) Fracture of both the thigh bones.
- (b) Injection of 1/2 c.c. of 1 : 10 formalin subcutaneously in the thigh.
- (c) Scald—By dipping the hindlimbs in boiling water for 0.05 minutes.

Hypophysio-portal circulation has been studied after injection of India ink. For the demonstration of neurosecretory substance, the brains have been perfused with Bouin's fluid and fixed in it and sections stained by Gomori's chrome-alum-haematoxylin method. For adrenal, formalin fixation has been done and frozen sections have been studied for Birefringence. Sections have been stained by Sudan IV and Haematoxylin and Eosin. Schultz test has also been applied.

*Hypophysio-portal circulation :—*

Popa and Fielding (1930) stated that blood was collected from the pituitary lobes and ascending along the stalk was distributed by a secondary capillary net in the hypothalamus. Wislocki and King (1936) and Wislocki (1937, 1938) confirmed the presence of these vessels but they were of the opinion that blood flowed from the median eminence towards the pituitary. Thus they contradicted the direction of the flow of blood as stated by Popa and Fielding. Houssay, Biassoti and Sammartino (1935) first described the hypophysio-portal circulation in a living toad. The direction was from the hypothalamus towards the ventral surface of the pars distalis. When these vessels were cut or burned, there was necrosis of an extensive area of the anterior part of the pituitary ; but because the pars intermedia, neurohypophysis and some portion of the anterior lobe received extra blood supply from the brain and the basilar artery, they escaped necrosis. Lascano-Gonzalez (1935) observed infarction of the pars distalis in toads after lesions of the hypothalamus



anterior to the hypophysis, but the neurohypophysis escaped. Subsequently the direction of the blood flow was confirmed by many to be from the median eminence towards the pars distalis (Green and Harris 1949, Morato 1939, Barnett and Greep 1951, Green and Harris 1947, Green 1951). Green (1951) stated that in frogs and toads the vessels after coming out of the median eminence formed portal vessels which supplied the pars distalis through a secondary capillary net. The importance of this vascular connexion was stated by Harris (1944) and Green and Harris (1947) for the proper functioning of the anterior pituitary. Green (1951) mentioned, "The hypophysio-portal circulation could conceivably control the activity of the pituitary in one of two ways, by regulating the blood supply to the gland or by means of one or more hormonal substances produced in the median eminence as the result of nervous activity and carried to the pars distalis there to exert an excitatory or inhibitory effect."

In this present investigation the hypophysio-portal circulation has been studied in the *Bufo melanostictus* and it has been found that vessels come out of the median eminence and form portal vessels which enter into the pars distalis and form secondary capillary net. The direction of flow is from the median eminence towards the pars distalis. When the stalk region is destroyed, there is infarction of the major portion of the pars distalis but no such change is found in the pars intermedia and neurohypophysis. In such preparations, after application of stresses (Fracture, Formalin injection, Scald) there is scantiness of Schultz positive areas and sudanophilic areas in the adrenal indicating increased activity. This means that the small number of surviving pars distalis cells is just sufficient to control the adrenal and the hypophysio-portal circulation is not important for the control of the hypophysis in the types of stresses used here. But it is also to be kept in mind that reunion of the severed portion by vascular granulation tissue is a possibility and actually it did happen in some of the preparations. Moreover in such preparations in *Bufo melanostictus*, obstruction by paper plate is difficult to perform and if at all it is done, it is very often misplaced.

#### *Stress and neurosecretory substance :—*

Neurosecretory substances could be traced from the nerve cells in the hypothalamus to the infundibulum along the axons. This was shown by Scharrer and Scharrer (1944), Palay (1945), Bargmann (1949, a, b). The material might be carried by the axoplasm current and Palay (1945) supported Weiss theory of axoplasmic migration. Passage of neurosecretory substances from hypothalamus to the posterior pituitary was shown by Hild (1953), Stutinsky (1952, 1954), Mazzi (1954), Benoit and Assenmacher (1954), Scharrer and Wittenstein (1952), and Drager (1950). Wagenvoort (1954), and Sloper (1954) found that after hypophysectomy in the rat, cat and dog, Gomori-positive granules were stored in the hypothalamic nuclei and fibers. This indicated that as the neuro-secretory substances could not reach the posterior pituitary because of its absence, they were stored in the hypothalamus and fibers. Sloper and Adams (1956) studied four patients hypophysectomized for the relief of malignant disease. They said that posterior pituitary principles were formed by the supraoptic and paraventricular nuclei, from where they passed



down the hypothalamo-hypophyseal tracts to accumulate in the posterior pituitary.

Rothballer (1953) found a characteristic response taking place in 3 stages after needle-prick in rats ; within one to two minutes after stimulation there was vasodilatation and the neurosecretory material moved towards the lumen of the blood vessels. Within 4-6 minutes there was vasodilatation with loss of neurosecretory material. The restoration process was started and completed within one to three hours. She considered that the same stimulus releasing the neurosecretory material gave rise to the discharge of ACTH and it was said, " These may be two parallel phenomena in the organisms response to noxious stimuli, but the possibility that they are more closely related deserves attention."

Okada *et al.* (1955) studied the relation of the neurosecretory cells to the third ventricle of *Rana catesbiana*. The results were as follows :—

(a) Cells of nucleus preopticus magnocellularis containing numerous neurosecretory granules, distribute from rostro-ventral to caudo-dorsal in the hypothalamus.

(b) Some long or short processes of them extend to the third ventricle through the ependymal layer and discharge their granules into the third ventricle.

(c) Some of fine nervous fibers containing granules or droplets at the subependymal layer, extend their ends into the ependymal cells, intra-ependymal spaces or third ventricle.

(d) Some of the neurosecretory cells discharge droplet like granules directly to the third ventricle through the intercellular spaces like dotted line or chain, and not through nervous processes.

(e) Some fibres of tractus praeopticohypophysius containing fine granules, reaching " Gliakammer," discharge granules into it."

Scharrer and Scharrer (1954) said about the delivery of the secretion in three ways :—

(a) Direct delivery in the blood path.

(b) Secretion in the ventricle.

(c) Transit along the nerve fibre.

Dawson (1952) found in the frog a fraction of the colloid ending in the blood vessels of the median eminence—which belonged to the hypothyseoportal system. It was observed that the transport of the neurosecretory substance to the anterior pituitary was along the vascular system. In frogs the neurosecretory substances in the bundle led into in between the cells of the pars intermedia.

In the present investigation, neurosecretory substances have been found in the neurosecretory cell groups in the hypothalamus of *Bufo melanostictus*. The study of seasonal variation is not yet complete as to give a definite conclusion regarding it. The passage of the neurosecretory substance is as follows :—

(a) Along the praeoptico-hypophyseal tract to the neurohypophysis. In experiments where this tract has been severed, there is damming of the substance in the proximal stump (48 hours).



(b) Into the third ventricle. All the different ways studied by Okada *et al.* (1955) in *Rana catesbiana* have not been found by me in *Bufo melanostictus*. I have found some and it seems to me that the chains of events are not separate but they are all parts of the ways of migration.

(c) The substance has been found to end in the blood vessels of the median eminence wherefrom they are carried to the pars distalis.

Within one hour of application of stresses (types mentioned before) there is congestion of the hypothalamus and pars nervosa in the early part and loss of neurosecretory substance from the hypothalamic nuclei and neurohypophysis. Replenishment of the substance occurs at varied intervals after one hour. Within the hypothalamic cells there are appearances of vacuoles and the neurosecretory granules and colloids surround them.

Similar types of depletion of neurosecretory substance from hypothalamic neurones and neurohypophysis has been observed by me in guinea-pigs and dogs after fractures and in man after fractures, other types of trauma, burns, and cholera. The loss of neurosecretory substance from the hypothalamus and neurohypophysis in man may be due to the agony during death apart from the disease or traumatic process itself.

#### STRESS AND HYPOPHYSIS IN BUFO MELANOSTICTUS

Congestive appearance of the hypophysis has been observed after application of the stresses (types mentioned before).

#### STRESS AND ADRENAL IN BUFO MELANOSTICTUS

In *Bufo melanostictus*, the adrenals are strips of golden yellow colour on the ventral surface of each kidney and at places partially embedded in kidneys.

Radu (1931) studied the brown coloured lipid droplets in the interrenal cells of *Rana* after treatment with osmium tetroxide. The cortical cells contain lipid drops in different quantities. The cortical lipid is doubly refractile to polarised light. Nileblue sulfate gives rise to rose colour. The cholesterin test is positive. The digitonin reaction gives complex doubly refractile crystals. Certain other histological characteristics have been stated.

Singer and Zwemer (1934) studied the adrenal histology of the living frog and compared with fixed, sectioned and stained tissues from the same animals similarly treated. There was change from granules to globules in the adrenal cells, but no change was found in the neighbouring kidney cells. There was a change in cytoplasmic structure after poisoning with mercuric chloride. "The pale yellow background begins to fade, and the yellow-brown granules enlarge until they appear as pale droplets or bubbles. At the end of half-an-hour, many of the cell groups have a frothy, water-white appearance. Finally, the greyish nuclei become distinct and the cell boundaries definitely outlined. At this state the granules still present are either coarser or uneven in size and distribution,



or are extremely fine, and the cytoplasm is very faint yellow or white, which gives the cell a watery appearance. A profile view of the growth of granules or bubbles reveals an extrusion phenomenon, the bubbles being projections into the lumina of the blood vessels."

In the present investigation within an hour after application of stresses the following changes in the adrenals of *Bufo melanostictus* have been observed.

(a) Pronounced congestion of the adrenals. The vessels on its surface stand out in prominence.

(b) Loss of sudanophilic substances.

(c) Presence of sudanophilic substances in neighbouring vessels.

(d) Schultz positive substance diminishes in the adrenal.

(e) Birefringent material diminishes.

(f) In the hæmatoxylin-eosin preparations there is vacuolar change in cortical cells as is found in human or other animal adrenals after different types of stresses.

From the adrenal findings it is evident that cortical lipides are discharged in response to stresses, and the lipides are helpful for the animal to tide over the critical period. The same conclusion can be made if we also consider the hypothalamic and hypophyseal changes as well. The changes have been previously described.

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## CHAPTER 10

### BRAIN MECHANISMS RESPONSIBLE FOR ACTH RELEASE IN THE TOAD—*BUFO MELANOSTICTUS* WITH A NOTE ON *CALOTES VERSICOLOR*

(1970)

Roy (1957) studied the hypophysio-portal circulation, hypothalamus, pituitary and adrenal of the *Bufo melanostictus* and changes in the hypothalamus after fracture and other stresses. Importance of the hypophysio-portal circulation was stressed and it was stated, "It is also to be kept in mind that reunion of the severed portion (of the stalk) by vascular granulation tissue is a possibility and actually it did happen in some of the preparations" after stalk section. The neurosecretory substances were noted in the median eminence from where they were carried to the pars distalis by hypophysio-portal vessels. Loss of neurosecretory substance was noted in the hypothalamic nuclei and neuro-hypophysis after stress and replenishment of the substance occurred at varied intervals after one hour.

Hypophysio-portal circulation was described by Roy (1958) in *Calotes versicolor* (Reptilia). The direction of flow of blood is from the median eminence and towards the pars distalis. Nerve fibres containing neurosecretory substance were found to end around the primary capillary net in the portion of the infundibulum which corresponds to the median eminence in higher vertebrates. "These findings help in the postulation of the idea that neurosecretory substance comes into the pars distalis through the hypophysio-portal vessels and a part contained in the substance stimulates the pars distalis to produce ACTH or gonadotrophin or other hormones." In *Calotes versicolor* the neurosecretory substance was found in the following situations (Roy, 1958) :—

- (a) in the neurosecretory cells of the hypothalamus as granules and along the axons of the cells.
- (b) in the extracellular spaces
- (c) towards the adjoining ventricle
- (d) upward extension from the hypothalamic level
- (e) neural lobe of the pituitary
- (f) in the richly vascularized median eminence region.

There was depletion of neurosecretory substance after stress and restorative phase occurred after some time.

The reviews mentioned above prove the importance of the hypophysio-portal vessels in the functioning of the pars distalis in amphibia and reptilia.

Importance of the median eminence and hypophysio-portal circulation was further shown by Roy (1960). In hypophysectomized toads and in



toads bearing ectopic autografts of the hypophysis no 17-OHCS was found in the blood. Measurable amounts of 17-OHCS were present in the blood of toads in which the hypophysis had been regrafted under the median eminence. Jacobsohn and Jorgensen (1956) observed no ACTH secretion from the ectopic pars distalis in toads utilizing another index *i.e.* survival time or abnormal moulting. van Dongen *et al.* (1966) observed the secretion of ACTH from the pars distalis deprived of hypothalamic contact in *Bufo* to be slight or absent.

Jorgensen (1968) attempted to localize structures in the brain of toads controlling ACTH secretion. This area was located in the middle and anterior region of the hypothalamus.

Roy (1964) observed no change in the gonads of the fish after pituitary stalk section. Hypophysectomized fishes with autografted pituitary in the anterior chamber of the eye showed atrophy of gonads but there was no atrophy of the anterior interrenal cells. Roy (1969) studied brain mechanisms responsible for ACTH release in the fish. It was observed that the dorsal telencephalon (primordium hippocampi, primordial general cortex, pars striatalis and primordium piriforme) has a checking influence over the pituitary-adrenal-axis. The ventral telencephalon including the primordium amygdalae and the nucleus preopticus has got a stimulatory control over the same axis. Positive response was obtained from the nucleus septalis medialis. The habenula is a modulating centre.

In this present investigation influence of the different areas of the brain on ACTH release from the pars distalis of the toad has been studied by stimulation and lesion experiments.

### MATERIAL AND METHODS

Adult *Bufo melanostictus* of both sexes have been used in this experiment under usual laboratory condition. Methods of stimulation and lesion of the brain are same as described by Roy (1969). The lesion of the ventral hypothalamus and median eminence was carried out through the roof of the mouth (Figs. 1, 2). Ether anaesthesia was used. Muscle relaxant was not used.

The animals were sacrificed by decapitation and plasma 17-OHCS content was estimated as described in 1969. The brains and the pituitaries were stained after Gomori's CAHP stain and paraldehyde fuchsin stain. The pituitaries were stained after Cleveland-Wolfe, and PAS-Orange. Sections of the brain were also stained after the methods of Kluver and Barrera.

Five *Calotes versicolor* have also been used for lesion experiments.

### RESULTS

*Stimulation experiments* (Figs. 3, 4) :—

Table I shows normal plasma 17-OHCS content/100 ml., appropriate controls of plasma 17-OHCS content for stimulation experiments of the



brain, and plasma 17-OHCS content after stimulation of different brain areas in toads. Table II shows the results of intergroup comparisons.

It is found that when the appropriate controls are compared to the normal, there is significant difference in plasma 17-OHCS level. Anaesthesia and surgery for placement of electrodes are stressing procedures leading to high plasma 17-OHCS level. Olfactory tract stimulation or stimulation of retina leads to significant rise in plasma 17-OHCS content. Stimulation of the primordium hippocampi lowers the plasma 17-OHCS level. Significant differences have not been observed on stimulating the primordium pallii dorsalis and primordium piriforme. Increased responses have been observed after stimulation of septal area, primordium amygdala, preoptic nuclear area, median eminence, dorsal and ventral hypothalamus.

TABLE I

Groups		Average	Range	No. of obs- ervations	S.D.	D.F.
A.	Normal Plasma 17-OHCS Content ..	2.500	0.9-4.0	10	0.905	9
B.	Appropriate controls of plasma 17-OHCS content for stimln.exp. of the brain.					
1.	Olf. t. stimulation ..	6.450	5.0-8.0	10	0.978	9
2.	Stimln. of retina ..	6.670	5.2-8.3	10	1.095	9
3.	Stimln. of p. hippocampi ..	6.270	4.8-8.1	10	1.090	9
4.	Stimln. of p. pallii dorsalis ..	6.500	5.3-7.9	10	0.836	9
5.	Stimln. of p. piriforme ..	6.950	5.2-8.5	10	1.033	9
6.	Stimln. of septal area ..	7.510	6.1-9.0	10	0.929	9
7.	Stimln. of p. amygdala ..	5.840	4.8-8.4	10	1.090	9
8.	Stimln. of pre-op. n area ..	6.710	5.8-9.0	10	0.941	9
9.	Stimln. of median eminence ..	5.920	4.1-8.3	10	1.245	9
10.	Stimln. of dorsal hypothalamus ..	6.340	4.7-7.8	10	1.054	9
11.	Stimln. of ventral hypoth. ..	6.770	6.0-8.6	10	0.791	9
C.	Plasma 17-OHCS content after stimulation of :					
1.	Olf. t. stimulation ..	8.380	5.8-11.2	10	1.685	9
2.	Stimln. of retina ..	8.800	6.7-10.9	10	1.284	9
3.	Stimln. of p. hippocampi ..	3.290	1.2-4.9	10	1.071	9
4.	Stimln. of p. pallii dorsalis ..	7.540	5.9-9.6	10	1.330	9
5.	Stimln. of p. piriforme ..	7.340	5.6-9.9	10	1.393	9
6.	Stimln. of septal area ..	9.110	8.0-12.1	10	1.229	9
7.	Stimln. of p. amygdala ..	8.760	6.2-12.6	10	1.820	9
8.	Stimln. of pre-op n. area ..	14.120	9.4-16.8	10	2.154	9
9.	Stimln. of median eminence ..	12.760	9.0-15.4	10	2.433	9
10.	Stimln. of dorsal hypoth. ..	8.260	5.6-11.0	10	1.630	9
11.	Stimln. of ventral hypoth. ..	15.470	10.1-18.0	10	2.297	9



TABLE II

Statistical table showing D.F. and values of 't' and their significance for different types of 'intergroup' comparisons

Group A vs Group B				D.F.	't'
1.	Normal vs. control olf. t. st.	..	..	18	— 9.404***
2.	Normal vs. control for st. of retina	..	..	18	— 9.287***
3.	Normal vs. control for st. p. hippo	..	..	18	— 8.396***
4.	Normal vs. control for st. p. palli dor.	..	..	18	— 10.282***
5.	Normal vs. control for st. p. piriforme	..	..	18	— 10.277***
6.	Normal vs. control for p. septal area	..	..	18	— 12.249***
7.	Normal vs. control for p. amygdala	..	..	18	— 7.472***
8.	Normal vs. control for pre-op. n. area	..	..	18	— 10.218***
9.	Normal vs. control for med. eminence	..	..	18	— 7.037***
10.	Normal vs. control for dor. hypoth.	..	..	18	— 8.747***
11.	Normal vs. control for ventral hypoth.	..	..	18	— 11.266***
Group B vs. Group C.					
1.	Control for olf. t. st. vs. after olf. t. st.	..	..	18	— 3.138**
2.	Control for st. of retina vs. after st. of retina	..	..	18	— 3.996***
3.	Control for st. of p. hippo vs. after st. of p. hippos.	..	..	18	— 6.131***
4.	Control for st. p. palli dor. vs. after st. p. palli dor.	..	..	18	— 2.092
5.	Control for st. p. piriforme vs. after st. p. piriforme	..	..	18	— 0.711
6.	Control for st. p. septal area vs. after st. septal area	..	..	18	— 3.292**
7.	Control for st. p. amygdala vs. after st. p. amygdala	..	..	18	— 4.358***
8.	Control for st. pre-op. n. area vs. after st. pre-op. n. area	..	..	18	— 9.973***
9.	Control for st. med. eminence vs. after st. med. eminence	..	..	18	— 7.918***
10.	Control for st. dor. hypoth. vs. after st. dor. hypoth.	..	..	18	— 3.127**
11.	Control for st. vent. hypoth. vs. after st. vent. hypoth.	..	..	18	— 11.328***

\* Significant at 5% level

\*\* Significant at 1% level

\*\*\* Significant at 0.1% level or more stringent level

TABLE III

Groups				Average	Range	No. of Observation	S.D.	D.F.
D. Plasma 17-OHCS content in controls for brain lesioned animals at different periods								
1.	24 hours	..	..	6.540	4.7-9.0	10	1.612	9
2.	7 days	..	..	3.100	0.8-5.4	10	1.424	9
3.	14 days	..	..	2.600	0.7-4.7	10	1.050	9
E. Plasma 17-OHCS content after lesion of p. hippocampi								
1.	24 hours	..	..	14.280	10.1-18.5	10	2.505	9
2.	7 days	..	..	12.800	8.9-16.9	10	2.315	9
3.	14 days	..	..	10.260	6.8-15.1	10	2.889	9
F. Plasma 17-OHCS content after lesion of septal area.								
1.	24 hours	..	..	5.110	3.5-7.4	10	1.183	9
2.	7 days	..	..	2.090	0.6-5.0	10	1.519	9
3.	14 days	..	..	1.890	0.8-4.2	10	1.100	9
G. Plasma 17-OHCS content after lesion of amygdala.								
1.	24 hours	..	..	4.210	2.7-6.5	10	1.341	9
2.	7 days	..	..	3.580	2.2-6.2	10	1.228	9
3.	14 days	..	..	2.950	1.5-4.9	10	1.081	9



Groups				Average	Range	No. of Observation	S.D.	D.F.
<b>H. Plasma 17-OHCS content after lesion of pre-op. n. area</b>								
1.	24 hours	..	..	2.050	0.5-4.4	10	1.175	9
2.	7 days	..	..	1.870	0.6-3.5	10	0.914	9
3.	14 days	..	..	1.780	0.5-3.9	10	1.053	9
<b>I. Plasma 17-OHCS content after lesion of median eminence.</b>								
1.	24 hours	..	..	1.600	0.4-3.1	10	0.929	9
2.	7 days	..	..	1.400	0.4-2.9	10	0.831	9
3.	14 days	..	..	1.440	0.5-3.0	10	0.746	9
<b>J. Plasma 17-OHCS content after lesion of dorsal hypothalamus.</b>								
1.	24 hours	..	..	4.070	2.3-6.2	10	1.385	9
2.	7 days	..	..	2.900	1.4-5.3	10	1.163	9
3.	14 days	..	..	2.470	1.5-4.6	10	0.917	9
<b>K. Plasma 17-OHCS content after lesion of ventral hypothalamus.</b>								
1.	24 hours	..	..	1.610	0.7-3.0	10	0.677	9
2.	7 days	..	..	1.300	0.3-2.5	10	0.714	9
3.	14 days	..	..	1.050	0.2-1.8	10	0.554	9

TABLE IV

Statistical table showing D.F. and values of 't' and their significance for different types of "inter-group" comparisons

Group A vs. Group D					't'	D.F.
1.	Normal vs. controls for brain lesion	..	..	24 hours	-6.882***	18
2.	Normal vs. controls for brain lesion	..	..	7 days	-1.125	18
3.	Normal vs. controls for brain lesion	..	..	14 days	-0.228	18
Group D vs. Group E						
1.	Controls for brain lesion 24 hrs. vs. lesion of p. hippo			24 hours	- 8.216***	18
2.	Controls for brain lesion 7 days vs. lesion of p. hippo			7 days	-11.292***	18
3.	Controls for brain lesion 14 days vs. lesion of p. hippo			14 days	- 7.890***	18
Group D vs. Group F.						
1.	Controls for brain lesion 24 hrs. vs. lesion of Septal area.			24 hours	1.803	18
2.	Controls for brain lesion 7 days vs. lesion of Septal area.			7 days	1.534	18
3.	Controls for brain lesion 14 days vs. lesion of Septal area.			14 days	1.479	18
Group D vs. Group G						
1.	Control for brain lesion 24 hrs. vs. lesion of amygdala			24 hours	3.519**	18
2.	Control for brain lesion 7 days vs. lesion of amygdala			7 days	-0.808	18
3.	Control for brain lesion 14 days vs. lesion of amygdala			14 days	-0.735	18
Group D vs. Group H						
1.	Controls for brain lesion 24 hrs. vs. lesion of pre-op n. area.			24 hours	7.037***	18
2.	Controls for brain lesion 24 hrs. vs. lesion of pre-op n. area.			7 days	2.303*	18
3.	Controls for brain lesion 14 days vs. lesion of pre-op n. area.			14 days	1.744	18



Group D vs. Group I				t	D.F.
1.	Controls for brain lesion 24 hrs. vs. lesion of med. eminence.	24 hours		8.415***	18
2.	Controls for brain lesion 7 days vs. lesion of med. eminence.	7 days		3.262**	18
3.	Controls for brain lesion 14 days vs. lesion of med. eminence.	14 days		2.857*	18
Group D vs. Group J					
1.	Controls for brain lesion 24 hrs. vs. lesion of dorsal hypothalamus.	24 hours		3.681**	18
2.	Controls for brain lesion 7 days vs. lesion of dorsal hypothalamus.	7 days		0.344	18
3.	Controls for brain lesion 14 days vs. lesion of dorsal hypothalamus.	14 days		0.295	18
Group D vs. Group K					
1.	Controls for brain lesion 24 hrs. vs. lesion of ventral hypothalamus.	24 hours		8.931***	18
2.	Controls for brain lesion 7 days vs. lesion of ventral hypothalamus.	7 days		3.571**	18
3.	Controls for brain lesion 14 days vs. lesion of ventral hypothalamus.	14 days		4.133***	18

\*Significant at 5% level

\*\*Significant at 1% level

\*\*\*Significant at 0.1% level or more stringent level

### Lesion experiments (Figs. 2, 5) :—

Table III shows plasma 17-OHCS content in controls for brain lesioned animals (anaesthesia and surgery for brain exposure), after lesions of p. hippocampi, septal area, p. amygdala, preoptic nuclear area, median eminence, dorsal hypothalamus and ventral hypothalamus at different periods. Table IV shows the results of intergroup comparisons. Anaesthesia and surgery for brain exposure stimulate the pituitary-adrenal-axis at 24 hours. However, at 7 and 14 days no significant difference from normals has been observed. After lesion of the p. hippocampi significantly higher levels of plasma 17-OHCS content have been observed at 24 hours, 7 and 14 days. Lesion of septal area does not show much difference. After lesion of p. amygdala significant fall has been observed only at 24 hours. Fall in plasma 17-OHCS content is significant after lesion of preoptic nuclear area at 24 hours and 7 days, after lesion of median eminence at 24 hours, 7 days and 14 days and after lesion of dorsal hypothalamus at 24 hours. Lesions of ventral hypothalamus show a significantly low level of plasma 17-OHCS at 24 hours, 7 and 14 days.

### Changes in neurosecretion :—

The median eminence was atrophic after lesion of ventral hypothalamus or preoptic cells. Neurosecretion in the nerve fibres were not found in the ventral hypothalamus distal to the level of section (in the group with ventral hypothalamic lesion). The neural lobe was atrophied and there was atrophy also of the preoptic cells. Regenerative features were, however, not observed in the second week.



Olfactory tract stimulation manifested with loss of neurosecretory material from the preoptic nuclei (magnocellular), the hypothalamo-hypophyseal tract and the neurohypophysis. Reaccumulation started from the terminal end towards the cell body and depended on the time interval between the cessation of stimulation and preparation for examination. With stimulation of the retina, loss of nsm was detected as in olfactory tract stimulation; but the magnitude of response was lesser. Grafting the pituitary into the hypophysiotrophic area (Fig. 6):—

Type of animals	Plasma 17-OHCS content
1. Hypophysectomized	1. Could not be measured
2. Hypophysectomized animals with grafts into the hypophysiotrophic area (HTA).	2. Could be measured (trace)
3. Hypophysectomized animals with grafts in HTA and the grafts maintained by vascularity from the hypophysis-portal vessels.	3. Normal

#### *Calotes Versicolor* :—

Removal of the hippocampus in *Calotes versicolor* leads to increased blood corticosterone level even in the second week after operation when the appropriate controls show normal values.

### DISCUSSION

Extrahypothalamic and hypothalamic controls over pituitary ACTH release are there in *Bufo melanostictus* and these have been proved by stimulation and lesion experiments. Stimulation of olfactory tract or retina leads to increased ACTH release. Primordium hippocampi has a checking influence. Septal area, primordium amygdala, preoptic nuclear area, median eminence, dorsal and ventral hypothalamus are all controlling centers for ACTH release. Brain mechanisms responsible for ACTH release in the fish have been described by Roy (1969). Similar control has been described for mammals by Egdahl (1960, 1962), Egdahl *et al.* (1958, 1959), Endrőczii *et al.* (1954), Endrőczii and Lissák (1960), Lissák and Endrőczii (1960), Mason (1958), Roy (1960, 1962, 1966, 1967, 1968) and others.

Jorgensen (1968) studied the importance of the hypothalamus regarding the control of corticotropic function in *Bufo bufo*. He said that pre- and post-chiasmatic section of the hypothalamus practically always had reduced corticotropin secretion from the hypophysis.

Stimulation and lesion experiments of the dorsal and ventral hypothalamus indicate that a diffuse area in the hypothalamus from the chiasmatic area to the posterior aspect of the hypothalamus exists which controls ACTH release in the toad. Similar observation has been made by Brodish (1964) for rats. Jorgensen (1968) came to the conclusion that from preliminary results it could be suggested that in amphibians the hypothalamic centers might be diffuse and overlapping.

Roy (1960) stated that in toads subjected to the removal of the pituitary from its normal situation and autografted on other parts of the brain,



17-OHCS in the peripheral blood could not be measured. Measurable amounts of 17-OHCS were found in toads with regrafting of the anterior lobe to the median eminence. Autografting of the pituitary to other parts of the brain did not include the medial basal hypothalamic region which has been called by Halasz *et al.* (1965) as hypophysiotrophic area (HTA). This area includes the arcuate nucleus, the ventral part of the anterior periventricular nucleus and the medial part of the retrochiasmatic area. However, in the present study some experiments have been carried out to elucidate these points. It has been observed that measurable amount of 17-OHCS in plasma could not be detected in hypophysectomized toads. Trace 17-OHCS could be measured in hypophysectomized animals with grafts into the hypophysiotrophic area. Plasma 17-OHCS content was normal in hypophysectomized animals with grafts in HTA and the grafts maintained by vascularity from the hypophysis-portal vessels. Therefore, it can be stated that this vascularity is essential for the normal functioning of the pituitary with respect of ACTH release. Stress response in such animals is to be noted.

There were changes in nsm in preoptic nucleus (magnocellular), hypothalamo-hypophysial tract, median eminence and neural lobe. Roy (1960) discussed these changes in different experimental conditions. With pituitary stalk section in *Bufo melanostictus* there was moderate to plenty neurosecretory material in the preoptic nucleus and plenty neurosecretory material in the preoptico-hypophysial tract near median eminence. There was moderate lipid store in the adrenals. Similar features were noted after lesion of median eminence.

In *Calotes versicolor* the hippocampus has a checking influence on the pituitary-adrenal-axis.

Neural pathways engaged in pituitary ACTH release in *Bufo melanostictus* have been mentioned in Fig. 7. The medial forebrain bundle connects the septal nuclei and the primordium hippocampi with the hypothalamic regions. Amygdaloid area is connected to the septal area. The preoptic and the dorsal hypothalamic area receives fibres from stria terminalis. The amygdaloid area and the primordium piriforme are connected with the preoptic and hypothalamic areas.

Ariens Kappers *et al.* (1936) said "The connections with the hypothalamus and the ventral thalamus are through a primitive fornix system from the archipallial regions of the cortex, and by way of the medial forebrain bundle from the septal regions and the tuberculum olfactorium."

Some of the neural pathways engaged in pituitary ACTH release in *Calotes versicolor* have been mentioned in Fig. 8. The area of the brain removed included fascia dentata and dorsal cortex (Ammon's pyramidal cells) of Ariens Kappers (1936) (Fig. 9).

## CONCLUSIONS

- (1) Olfactory tract stimulation or stimulation of retina leads to significant rise in plasma 17-OHCS content of *Bufo melanostictus*.
- (2) Primordium hippocampi has got a checking influence over the pituitary-adrenal-axis.



(3) Septal area, primordium amygdala, preoptic nuclear area, median eminence, dorsal and ventral hypothalamus are all controlling centers for ACTH release in *Bufo melanostictus*.

(4) Changes in neurosecretory material were observed in the preoptic magnocellular nuclei, hypothalamo-hypophyseal tract, median eminence and neural lobe of the pituitary after stimulation or lesion experiments.

(5) Corticotrophin releasing factor (CRF) is possibly engaged in the release of ACTH from the pars distalis of the toad.

(6) Plasma 17-OHCS could be measured (trace) in hypophysectomized animals with grafts into the hypophysiotrophic area. Normal values were found in animals where the pituitary grafts were maintained by vascularity from the hypophysio-portal vessels.

(7) In *Calotes versicolor* the hippocampus has a checking influence over the pituitary-adrenal-axis.

(8) The pathways engaged in ACTH release in *Bufo melanostictus* and *Calotes versicolor* have been stated.

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## CHAPTER—11

### THE HYPOPHYSIO-PORTAL CIRCULATION, HYPOTHALAMUS, PITUITARY AND ADRENAL IN *CALOTES VERSICOLOR* AND CHANGES IN HYPOTHALAMO-PITUITARY- ADRENAL-AXIS IN FRACTURE AND OTHER FORMS OF STRESSES (1958)

#### *Materials and Methods of the Works :*

#### TYPE OF STRESSES :

1. Fracture of both the thigh bones,—Examination started 1 hour after fracture.
2. Scald—by putting the hindlimbs in boiling water for 0.05 minutes.
3. Ether anaesthesia for 15 minutes.

India ink preparation has been used for the study of the hypophysio-portal circulation. Neurosecretory substance has been studied after perfusion and fixation of the brain with Bouin's fluid and staining the section by Gomori's chrome-alum-haematoxylin-phloxine method. Histological examination of the adrenals comprises the following :

- (a) Sudan IV stain.
- (b) Haematoxylin and eosin stain.

#### *Hypophysio-portal Circulation :*

The importance of the hypophysio-portal circulation has been stressed by Harris (1944), Green and Harris (1947) and Green (1951). This link maintains the anterior pituitary in proper functioning condition.

The *Calotes versicolor* has got well-marked median eminence and the primary capillary net of the portal vessels are partly within it and partly on the surface of it. Through the pars terminalis the portal vessels enter into the pars distalis where they break up into secondary capillary net. On the surface of the median eminence there are basophilic cells akin to the pars tuberalis. There is well developed neural lobe and pars intermedia. The direction of flow of blood in the hypophysio-portal vessels is from the median eminence towards the pars distalis. Nerve fibres containing neurosecretory substance are found to end around the primary capillary net in the portion of the infundibulum which corresponds to the median eminence in higher vertebrates. These findings help in the postulation of the idea that neurosecretory substance comes into the pars distalis through the hypophysio-portal vessels and a part contained in the substance stimulates



the pars distalis to produce ACTH or gonadotrophin or other hormones.

The hypothalamus, median eminence region and the hypophysis show marked congestion after the stresses used in this experiment.

*The Hypothalamus and Neurosecretory Substance :*

The neurosecretory substance is prepared in the hypothalamic cells and transported to the posterior pituitary—Hild (1953) ; Stutinsky (1952, 1954) ; Mazzi (1954) ; Benoit and Assenmacher (1954) ; Scharrer and Wittenstein (1952) ; Drager (1950) ; and Bargmann (1954). The material is carried by an axoplasmic current. The neurosecretory cells in the hypothalamus are richly vascularised and functional control of these cells by changes in the blood and pouring out of their secretory product directly into the blood path are possibilities. That the neurosecretory product also reaches the anterior pituitary and controls its function has been mentioned by many and specially Harris (1944), Green (1951), Green and Harris (1947), de Groot (1952), Assenmacher and Benoit (1953*a, b*), Benoit and Assenmacher (1951*a, b, c, d*, 1952, 1954). The neurosecretion is also poured into the adjoining ventricle from the neighbouring hypothalamic cells. Such has been mentioned by Hild (1951).

*Changes in the Neurosecretory cells in different stages of activity :*

Frequently empty vacuoles have been observed in the cytoplasm of neurosecretory cells (E. Scharrer, 1933 ; Oliveira E. Silva, 1937 ; Bargmann, Hild, Ortmann and Schiebeler, 1950 ; Hild, 1951). Scharrer and Scharrer (1954) have mentioned that as such kind of vacuoles are not present in majority of animals, so it is questionable whether they play any important part in the neurosecretory process. A cell with granules can be taken as active and one without granules as resting phase.

In the *Calotes versicolor* the neurosecretory substance has been found in the following situations :

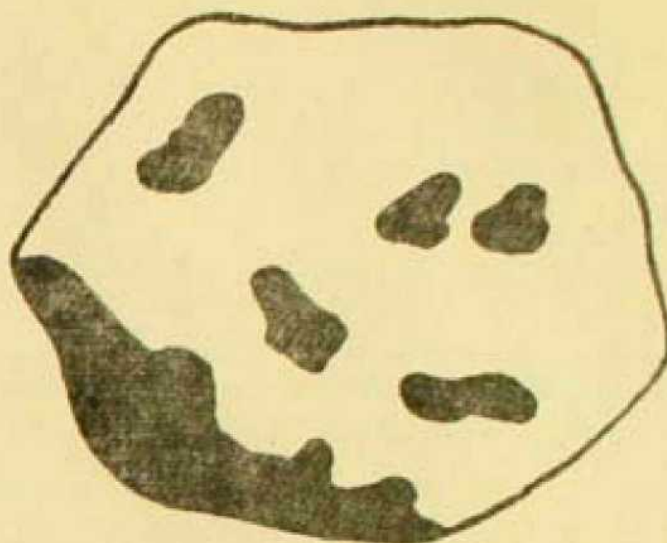
- (a) in the neurosecretory cells of the hypothalamus as granules and along the axons of the cells.
- (b) in the extra-cellular spaces.
- (c) towards the adjoining ventricle.
- (d) upward extension from the hypothalamic level.
- (e) neural lobe of the pituitary.
- (f) in the richly vascularised median eminence region.

After fracture and other stresses there was congestion followed by depletion of neurosecretory substance from the above mentioned areas. The restorative phase occurred after sometime. The vacuolar change in the neurosecretory cells is most commonly met with after stress. The pattern of change in the cell group is mostly uniform but some cells may show different stages of activity.



## Chapter 11

*Ventral aspect*



*Dorsal aspect*

*Diagrammatic Representation of the Suprarenal gland in Calotes versicolor to show adrenal and the inter-renal components. Areas occupied by adrenal elements are shown black and those occupied by inter-renal elements are white.*

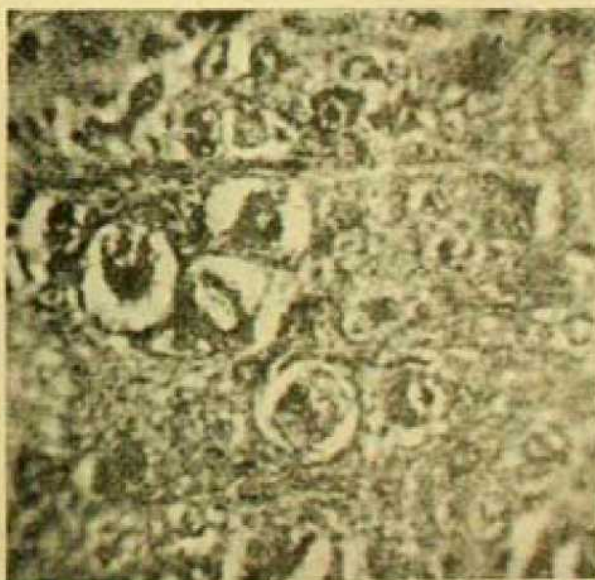


Fig. 2. Photomicrograph showing the neurosecretory cells in the hypothalamus of *Calotes versicolor*. Majority of the cells show vacuoles within them (CAHP stain Gomori).

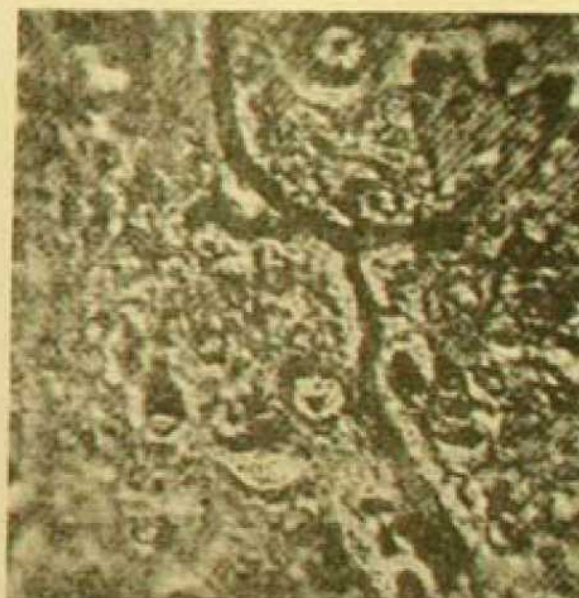


Fig. 3. Photomicrograph showing the neurosecretory cells and axons in the hypothalamus of *Calotes versicolor*. The upward extension is towards the adjoining ventricle and the downward one is towards the median eminence (CAHP stain of Gomori).



### *Adrenal and Stress in Calotes Versicolor :*

The Reptilian suprarenal is a transition form between the Amphibian suprarenal with the adrenal and inter-renal portions lying together and that of the birds with thorough mixing of both the portions in a disorganised way. In the *Calotes versicolor* the adrenal element lies in the dorsal aspect of the gland ; but scattered groups or solitary adrenal cells are found in the gland. The adrenal cells are big with a darkly stained nucleus. The inter-renal cells are small and contains lipid. The gland is very vascular. Glandular hypertrophy occurs in summer whereas during winter the reverse is true. Stress (types as mentioned before) leads to congestion of the organ and loss of sudanophilic substance from the inter-renal cells. In the haematoxylin-eosin preparation there is vacuolar change in the inter-renal cells.

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## CHAPTER—12

### ENVIRONMENT, STRESS AND ADRENOCORTICAL RESPONSE

( 1960 )

"The environmental factors which influence the endocrine organs may be "physical" in the sense of an alteration in hours of daylight, or "social" in the sense of stimulation deriving from the group of which an animal is part. Some changes appear to be mediated through the sensory modality of touch—for example, the stimulation arising from coitus (or pseudo-coitus) which, in the rabbit or ferret, results in ovulation ; stimulation of the reproductive tract which in the rat leads to pseudo-pregnancy ; the pressure-pattern of the eggs which determines the number of eggs that are laid by a bird in a single clutch ; and the nipple-stimulation on which the maintenance of milk-secretion at least partly depends. Others are mediated through the visual and auditory modalities, and comprehend such reactions as the increased secretion of adrenaline following a frightening noise ; the induction of breeding activity in an anoestrous ferret as a result of exposure to illumination during the hours of darkness ; and the *egg-laying* of an isolated pigeon which is shown its image in a mirror. ....The chain of events in reactions of this kind is presumably (a) stimulation of one or more sets of receptors ; (b) the transmission of afferent impulses to the thalamus, hypothalamus and cortex ; (c) the initiation or modification of hypothalamic activity by thalamus and cerebral cortex ; (d) excitation of adenohypophysis ; and finally (e) differential activation of peripheral endocrine organs." [Zuckerman (1952)]. Thus Zuckerman stated the importance of environmental factors in influencing the endocrine organs. Hooded ferret and mare did not respond to additional light (Bissonnette, 1936 ; Burkhardt, 1947). Thomson (1951) designed some experiments to find out whether the optic nerve fibres or the retinal blood vessels or the ciliary nerves and vessels are responsible for the light-induced oestrus. Ferrets with lesions of the optic nerves or those with lesions of the retinal blood supply did not come into oestrus in response to added illumination. With both these procedures there was degeneration of the ganglion cell layer of the retina. Thus retina is the important site for the transference of the light stimuli to the pituitary in ferrets and the initial mechanism is a nervous one.

In birds, there are receptors other than the retina which receive and transmit light stimuli to the pituitary (Benoit and Assenmacher, 1953-a, 1953-b). Exposure of 15 hours of light per day for three weeks to immature *drake* will lead to good testicular development. Section of the optic nerves or enucleation of the eyeballs will not prevent the stimulation of the testes totally. The pituitary should be present for this response. They have postulated the presence of the photoreceptors in the orbital region of the duck. Direct illumination of the hypophysial region or of the hypothalamus (in the region of the supraoptic nuclei) or of the rhinencephalon will produce testicular growth. The response after illumination of the optokinetic area was negative. The translucent nature of the skull



allowed a good amount of light to enter into the deeper areas of the brain. Deeper structures of the brain are sensitive to the whole of the visible light spectrum.

Oksche, Laws and Farner (1958) found that the nerve loops in the median eminence of the white crowned sparrow (*Zonotrichia leucophrys gambelli*) are filled with neurosecretory material when exposed to 8 hours of illumination daily. No testicular growth was observed in these animals. When the photoperiod was increased to 20 hours daily for 5, 10, or 20 days there was rapid testicular growth which was unseasonal. The median eminence in these animals had only scanty neurosecretory material.

Clark, McKeown and Zuckerman (1939) found that neither the integrity of the visual cortex nor the mid-brain is essential for the light impulses from the retina and it was suggested that the response depended on impulses passing either to the ventral nucleus of the lateral geniculate body or the subthalamus by way of accessory optic tracts.

Frey (1947) found the evidence for the existence of an optico-hypothalamic tract and an optic hypothalamic center in the guinea-pig. In crustaceans, light stimulus reaches the brain through the compound eye and the hormone is released into the circulation and this leads to contraction or expansion of the pigments in various types of chromatophores.

Askin and Vidarskaja (1956) thought that the diencephalon plays its part in the mechanism regulating the influence of light on the function of the adrenal cortex.

Wolfson (1959) states that in birds, "the hypothalamus is the primary target organ of the external factor, the daily durations of light and darkness, and that the interaction between the external factor and the hypothalamus results in the occurrence of spring migration and breeding periods at the proper time of the year."

Optical stimuli set up an impulse over an optico-hypothalamico-hypophysial pathway which causes secretion of water balance factors from the neurohypophysis of frogs and albino rats (Boyd *et al.*, 1940, 1943). They mentioned the pathways of reflexes originating in the eyes. The primary centres are :—(a) lateral geniculate bodies and (b) superior colliculi.

The impulses travel to the lateral geniculate body and from there to the supraoptic nuclei *via* the dorsal supraoptic commissure. From the superior colliculi, impulses may pass along the tectospinal tract, the medial longitudinal fasciculus and the tectocerebellar tracts. The tectospinal tract makes various connections possible, for example from the red nucleus to the tuberal nuclei of the hypothalamus. Through the medial longitudinal fasciculus, many other pathways are possible.

Knoche (1956) proved that there is a tract of non-medullated fibres connecting the retina with the hypothalamus. Some of the fibres end in the vegetative nuclei of the diencephalon and some pass through the connecting stalk and end in the posterior lobe of the hypophysis.

Jefferson (1940) described the subcortical connections of the optic tracts of the ferret. It appeared that the ferret did not possess a "dorsal hypothalamic root" or either of the accessory optic tracts. The central



nucleus of the lateral geniculate body did not receive any optic connections....Possibly the optic fibres ended in the pretectal region although these terminations were not demonstrable in the ferret material available. There may be no special pathway mediating the gonadal response of the ferret to retinal stimulation. It may be an indirect response to the changes in total bodily activity of the animal, which are primarily induced by such stimulation.

Brown-Grant *et al.* (1954) found thyroid inhibition after varying the conditions of environmental lighting.

The postnatal development of the neuron with regard to its mass was proved by Brattgard (1952) investigating the ganglion cells of the retina. The presence of normal light-path near the cell mass results in volume unit in the early (postnatal) period to 100%.

TABLE I

Showing the total dry weight of carnoy fixed retinal ganglion cells in different ages.

Weight in  $10^{-9}$  mg./ $\mu^3$

11 day old rabbit	10 week old rabbit	8 month old rabbit
$0.42 \pm 0.016$	$0.78 \pm 0.013$	$0.98 \pm 0.038$

After Brattgard and Hyden, 1954.

The influence of adequate stimulus on the development of the retinal ganglion cells from birth to growing stages is shown in Table II

TABLE II

Showing weight in  $10^{-9}$  mg./ $\mu^3$

Rabbit in usual day light for 11 days	Rabbit in usual day light for 10 weeks	Rabbit born in darkness and in darkness for 10 weeks	Rabbit born in darkness and kept in darkness for 10 weeks and then for 3 weeks in daylight
$0.42 \pm 0.016$	$0.78 \pm 0.013$	$0.16 \pm 0.012$	$0.58 \pm 0.021$

It will be seen that light is an important stimulus for the growth of the retinal ganglion cells.

Scharrer (1959) stated "There is a growing body of information which indicates that in invertebrates and vertebrates alike, the brain serves as the centre of integration of nervous, chemical, physical, and other stimuli resulting from environmental changes as well as from the internal milieu of the organism. The common final path by which these stimuli reach the endocrine system appears to be the neurosecretory cell which is capable of receiving nervous impulses and transforming them into endocrine



activity. That the hypothalamus plays an important part in this process is no longer merely assumed, but has been generally accepted as a fact."

Neurosecretory activity in rats kept under conditions of continuous light or darkness has been studied by Fiske and Greep (1959). Fortier (1950) found the release of an increased amount of ACTH when rats were subjected to sudden intense light or sound.

In the present investigation light has been taken as one of the environmental factors and its influence on the brain of the *Palaemon carinus* and on the hypothalamo-pituitary-adrenal axis in toads, guineapigs and dogs has been studied. The effect of sound as stimulus has also been studied. Photographic studies have been made in order to find out whether deeper penetration of light occurs in invertebrates and vertebrates. The effect of fracture and burn has also been studied in different experimental preparations.

**MATERIALS AND METHODS**—In the *Palaemon carinus* the eye-stalks were removed and these served as controls ; after exposure to strong light, the brain and the ganglia were examined by fixation in Bouin's fluid and staining after Gomori's chromealum-haematoxylin-phloxine method.

In the experiments with toads (*Bufo melanostictus*) the plan was as follows :—(a) 5 toads with exposure to constant illumination from 100 w-bulbs ; (b) 5 toads kept in darkness ; and (c) 5 toads kept under normal laboratory conditions.

The experimental period lasted for two weeks, at the end of which the toads were sacrificed by decapitation and heparinized blood was taken for 17-Hydroxycorticosteroid estimation after the method of Silber and Porter (1954). The brain and the pituitary were fixed in Bouin's fluid and the sections were stained by Gomori's CAHP staining method. The adrenals were fixed in formalin and frozen sections were stained with Sudan III and some sections of adrenals and pituitary were stained with haematoxylin and eosin. Estimation of 17-Hydroxycorticosteroids was performed in the peripheral blood, one hour after fracture of the right femur and scald to the right hind limb. Adrenal venous effluents were collected and 17-OHCS content was noted. Similar procedures as mentioned above was done in hypophysectomized, pituitary stalk sectioned toads and toads with lesions of the median eminence.

The experiments with guinea pigs were planned as described for toads. Male guinea pigs of 400 to 450 g. in weight were used. Five guinea pigs were kept in constant illumination after sectioning the optic nerves. Intense sound was also used as a stimulus.

Male dogs of 10-15 kg. in weight were used in these experiments. Ether or pentobarbital sodium was used as the anaesthetic agent. The right adrenal vein was cannulated for collection of the adrenal venous effluent in the conscious animal.

Exposure to strong illumination, intense sound stimuli, fracture of the right femur and burn injury to the right hind limb were used as stimuli in normal, hypophysectomized, pituitary-stalk-sectioned and hypothala-



mectomized dogs and dogs with lesion of the median eminence. Light stimulus was also tested in dogs with lesion of the optic nerves. Fracture and burn trauma were also studied in spinal transected (C7) dogs and in dogs after removal of the brain leaving only pituitary, pons, medulla, cerebellum and mid-brain intact (isolated pituitary experiment).

The hypothalamus and the pituitary with the pituitary stalk (where present) were fixed in Bouin's fluid and sections stained after Gomori's CAHP staining method. The adrenals were fixed in formalin and sections were stained with Sudan III and haematoxylin and eosin.

Photographic studies were made by placing the papers at the base of the brain of the *Palaemon carinus*, *Bufo melanostictus*, Bekt fish (*Lates calcalifer*), guinea pigs and rabbits and then exposing to different light. This was done with a view to knowing whether encephalic penetration of light occurred or not.

*Bufo melanostictus*—Five toads were used in each group ; the average values are given in Table III below :—

TABLE III  
Showing the average values for *Bufo melanostictus*

	Usual laboratory condition	Constant illumination	Kept in dark	Fracture	Scald
	17-OHCS	mcg.	per	100 ml.	Plasma
Control	3.3	15.5	1.2	17.2	25.3
Hypophysectomized	Nil	Nil	Nil	Nil	Nil
Pituitary stalk sectioned	1.8	2.3	2.2	12.1	18.3
Median eminence lesion	1.2	2.2	2.3	3.4	5.4



Guinea pigs—Five animals were used in each group, and the average values are tabulated below :—

TABLE IV  
Showing the average values for guinea pigs

	Usual labora- tory condition	Constant illumi- nation	Kept in dark	Sound stimuli	Fracture	Burn	ACTH 0.5 I. U. injected I. V.
	Plasma	17-OHCS	meg.	per	100	ml.	
Control	26.3	49.2	12.5	52.3	54.3	92.5	105
Hypophy- sectomized	7	8.1	6.9	7.6	7.3	9.4	26
Pituitary stalk sectioned	15	17.3	14.7	16.2	26.8	68.5	92.3
Ventral hypo- thalamus and median eminence lesion	9	11.3	8.7	10.6	15.3	28	21
Lesion of optic nerves	20.4	2.5	21.3	..	..	..	112
High spinal transection	22.5	..	..	..	41.3	71.2	92

Dogs—Four animals were used in each group and average values given in Table V.

TABLE V  
Showing the average values for dogs

	Usual labo- ratory condition	Exposure to strong light 2 hrs.	Sound stimulus 15 mts.	Fracture 1 hr. after	Burn 15 min. after	ACTH 0.5 U/Kg.
17—OHCS output meg. per minute—adrenal venous blood.						
Control	4.2	8.4	8.1	15.3	25.1	20.2
Hypophy-sectomized 48 hrs. after Total	0	0	0	0	0	4.3
50%	1.5	1.2	0.5	8.3	15.7	10.8
Pituitary stalk sectioned	2.1	2.3	1.8	9.4	18.3	12.7
Anterior median eminence lesion (within 24 hrs.)	1.2	1.8	1.7	6.6	10.4	10.3
Ventral hypo-thalamec- tomy with median emi- nence lesion (within 24 hrs.)	1.4	1.6	1.3	7.2	13.2	11.2
Lesions of optic nerves	3.2	4.4	..	..	..	..



Dogs—Four animals were used in each group and the average values are given below :—

TABLE VI  
Showing the average values for dogs—(Contd.)

	Usual laboratory condition	Fracture 1 hr. after	Burn 15 minutes after	ACTH 5U/Kg.
Adrenal venous 17-OHCS output—Gamma/minute.				
Spinal transected (O7)	2.9	10.7	18	20.3
Removal of brain leaving only pituitary, pons, cerebellum, medulla. (Isolated pituitary experiment).	3 hours after operation			
	7.6	23.1	35.6	36.3

Results—Biochemical—*Bufo-melanostictus* :—The 17-OHCS in the peripheral blood of toads in nonstressed condition is 3.3 gamma per 100 cc. plasma. With constant illumination it rises to 15.5 gamma per 100 cc. but when the toads are kept in the dark, it is 1.2. Blind toads exposed to constant illumination showed a small rise in 17-OHCS content. Fracture and scald caused a rise in 17-OHCS and the maximum rise was with scald. In one of the control experiments, during the collection of blood after decapitation, the cut head with the parotid glands and the secretions accidentally fell into the collecting pot. The head was removed immediately and on analysis the plasma 17-OHCS content was found to be very high (about 5 times the normal value). Possibly the parotid secretion added to the positive Porter-Silber reaction. In the hypophysectomized toads 17-OHCS could not be found in the blood under any of the experimental conditions. Pituitary stalk section could inhibit the adrenocortical response due to constant illumination but it could not do so after fracture and scald and the response was lesser in magnitude in comparison to that found in normal toads after stress. Lesions of the median eminence could block the response to constant illumination and there was a very small rise after fracture and scald. In toads subjected to the removal of the anterior pituitary from its normal situation and autografted on other parts of the brain, 17-OHCS in the peripheral blood could not be measured. Measurable amounts of 17-OHCS were found in toads with regrafting of the anterior lobe to the median eminence. 17-OHCS in the adrenal venous blood was about 10 times higher than that found in peripheral blood. Seasonal variations in blood 17-OHCS content have been noted in *Bufomelanostictus*.

Guinea-pigs—17-OHCS content of the peripheral blood of guinea-pigs under usual laboratory conditions is 26.3 gamma per 100 cc. plasma. 17-OHCS is high in animals kept in constant illumination and it is low when kept in darkness. Intense sound leads to good adrenocortical response as evidenced by a rise in 17-OHCS content. Fracture and burn



(right hind limb burned by naked flame or right femur fractured) lead to increased adrenocortical response. With ACTH the 17-hydroxycorticosteroids were 105 gamma per 100 cc. plasma. After hypophysectomy, constant illumination did not lead to any appreciable rise in 17-OHCS content and a similar feature was noted after different types of stressing procedures. Hypophysectomized animals showed a low 17-OHCS content in the blood (7 gamma per 100 cc. plasma). ACTH in such animals lead to a rise in 17-OHCS content but it never approximated to the value noted in normal animals treated with ACTH. In pituitary stalk-sectioned animals the 17-OHCS content in the peripheral blood was low in comparison to normal animals. Constant illumination and intense sound stimuli do not lead to increased adrenocortical response. Fracture and burn lead to increased adrenocortical response but the magnitude is less than the response noted in control animals subjected to stress. ACTH led to increased adrenocortical response in pituitary-stalk-sectioned animals. Animals having lesions of the optic nerves when exposed to constant illumination showed very small rise in 17-OHCS content. Spinal transected animals exposed to the stress of fracture and burn showed increased adrenocortical response but the magnitude of response was less than that found in control animals subjected to similar stress. ACTH response was good in such animals. Guinea-pigs having lesions of the ventral hypothalamus and median eminence behaved differently. These were acute experiments. The 17-OHCS content in the peripheral blood in such animals kept in usual laboratory condition is very low (9 gamma per 100 cc. plasma). Constant illumination or intense sound is not a good stimulus for increased adrenocortical response in such animals. With fracture and burn there is very small rise in 17-OHCS content compared to the response noted in control animals. ACTH leads to very small rise in 17-OHCS content. Diurnal variations in plasma 17-OHCS content was noted in guinea-pigs but such variation was not found in animals with lesion of the anterior median eminence.

Dogs—17-Hydroxycorticosteroids were measured in the adrenal venous blood and the values presented as output in gamma per minute. Control dogs in the basal non-stressed condition of the body had the adrenal venous output of 17-OHCS as 4.2 gamma per minute. There were slight diurnal variations but during the daytime animals might show high values all on a sudden. Such sequence was lost in animals either with lesions of the anterior median eminence or with ventral hypothalamectomy and anterior median eminence lesion. The 17-OHCS output was high with exposure to strong light or sound stimulus. After lesion of the optic nerves, exposure to strong light showed no rise in 17-OHCS output. Fracture and burn led to a quick rise in 17-OHCS output. With ACTH the 17-OHCS output is 20.2 gamma per minute. Totally hypophysectomized dogs (48 hours) showed almost zero values in 17-OHCS output. With 50% hypophysectomy the values were low but increased response to the stress of fracture and burn and response to ACTH was noted. Pituitary stalk-sectioned animals did not show increased adrenocortical response after exposure to strong light or sound stimulus but such animals showed increased adrenocortical response after the stress of fracture and burn but the magnitude of response was less in comparison to that found in



the control animals subjected to similar stress. With ACTH, there was increased adrenocortical response. The 17-OHCS output in animals with lesions of the anterior median eminence was 1.2 mcg. per minute. Exposure to strong light or sound stimulus did not lead to increased adrenocortical response. With fracture, burn and ACTH there was increased response but the magnitude was lower than that found in control animals subjected to similar procedures. Ventral hypothalamectomy with median eminence lesion showed similar feature. Spinal transected dogs showed increased 17-OHCS output after fracture, burn and ACTH. In the isolated pituitary experiments, the basal level of 17-OHCS output was high and such animals behaved differently to the stress of fracture, burn or after ACTH injection with an increase in 17-OHCS output and the figures were highest in the dog series.

B. Histological—*Palaemon carcinus* :—*Palaemon carcinus* exposed to strong light showed hyperactivity in the brain cells and in the ganglia. When the eye-stalks were removed, exposure to strong light showed a depletion of neurosecretory material and vacuolation in the brain cells and the ganglia. This showed that light penetrated the deeper structures and activated the nervous system.

TABLE VII

Showing changes in neurosecretory material and adrenal lipid store in  
*Bufo-melanostictus* under different conditions

Experimental condition	Pre-optic nucleus	Preoptico-hypophyseal tract near median eminence	Neuro-hypophysis	Adrenal lipid store
Neurosecretory material				
Usual laboratory condition	M to P	M	M to P	Good lipid store
Kept in darkness	P	M	M	"
Constant illumination	S	S	M	D
Blind toads	M to P	M	M to P	Good lipid store
Blind toads and constant light	M	M	M	Slight D
Fracture	S	S	S	D
Scald	S	S	S	D
<i>Hypophysectomized (H)</i>	M	P	—	Moderate lipid store
H and constant illumination	M	P	—	"
H and kept in dark	M	P	—	"
H and fracture	M	P	—	"
H and scald	M	P	—	"
<i>Experimental conditions</i>				
Pituitary stalk section (S.S)	M to P	P	—	"
S.S. and constant illumination	M	P	—	"
S.S. and kept in dark	M	P	—	"
S.S. and fracture	S	S	—	D
S.S. and scald	S	S	—	"
<i>Median eminence lesion (M.E.L.)</i>				
M.E.L. and const. illumination	M to P	P	—	Moderate lipid store
M.E.L. and kept in dark	M			"
M.E.L. and fracture	M to P			"
M.E.L. and scald	M			"

P = Plenty, M = Moderate, S = Scanty, D = Depletion.



Hypophysio-portal circulation, hypothalamus, pituitary and adrenal of the *Bufo melanostictus* and changes in the hypothalamus, pituitary and adrenal after fracture and other stresses were studied by Roy (1957).

TABLE VIII

Showing the results in Guineapigs under various experimental conditions

Experimental conditions	Supra-optic nucleus	Supraoptico-hypophysial tract near median eminence	Neuro-hypophysis	Adrenal lipid store
	Neurosecretory material			
Usual laboratory condition	M to P	M	M to P	Good
Kept in dark	P	M	M	"
Constant illumination	S	S	M	D
Lesion of optic nerves and light	S	S	M to P	Moderate
Intense sound	S	S	S	D
ACTH	M	M	M	D
Fracture	S	S	S	D
Burn	S	S	S	D
<i>Hypophysectomized (H)</i>	M	P	..	Moderate
H and kept in dark	M	P	..	"
H and constant illumination	M	P	..	"
H and intense sound	M	P	..	"
H and fracture	M	P	..	"
H and burn	M	P	..	"
H and ACTH	M	M	..	D
<i>Pituitary stalk sectioned (S.S.)</i>	M to P	P	..	Moderate
S.S. and kept in dark	M	P	..	"
S.S. and intense sound	M	P	..	"
S.S. and fracture	S	S	..	D
S.S. and burn	S	S	..	D
S.S. and ACTH	M to P	P	..	D
<i>Median eminence lesion (MEL)</i>	M to P	P	..	Moderate lipid store
MEL and kept in dark	M to P	..	..	"
MEL and constant illumination	M	..	..	"
MEL and intense sound	M	..	..	"
MEL and fracture	M	..	..	"
MEL and burn	M	..	..	"
MEL and ACTH	M	..	..	"
<i>High spinal transection (S.T.)</i>	M to P	M	M to P	Good
S.T. and fracture	S	S	S	D
S.T. and burn	S	S	S	D
S.T. and ACTH	M	S	M	D

The anterior pituitary showed congestion and increased number of basophils after stress, light and sound stimuli. There were vacuolation in the basophil cells and evidences of increased cellular activity. Increased mitosis was seen in the pituicytes during stress and constant illumination, neurosecretory material in the pituicytes was scanty after application of stress, constant illumination or intense sound stimuli. In the dorsolateral hypothalamic area of guineapigs there were neurosecretory cellular formations having a different tinctorial property. The neuro-



secretory material was acidophilic and was depleted during stress. The same cycle of neurosecretory function continued in these cells as noted in the supraoptic nuclei. A detailed study of neuro-secretion and changes in the pituitary and adrenal was made by Roy (1959-b) in guinea pigs and dogs under different experimental and surgical conditions. When newborn guinea pigs were kept in constant light there was growth of neurosecretory cells with elevated 17-OHCS content in the peripheral blood. When such animals were kept in darkness, the neurosecretory cells did not mature properly and there was a diminished load of neuro-secretory material in them. The 17-OHCS content of the peripheral blood was low. This shows the influence of light in the maturation of the hypothalamic nuclei and also on the pituitary-adrenal axis.

**Dogs**—The neurosecretory phenomenon is identical to that observed in guinea pigs under different experimental conditions. Hypophysectomized dogs had atrophic adrenal glands. ACTH response was not noted. Partially hypophysectomized (50%) dogs had adrenal glands where there was some atrophy and which responded to stimuli and ACTH. Dogs with pituitary stalk-section had adrenals which responded to fracture and burn and ACTH but which did not respond to strong light or sound stimulus. Dogs with ventral hypothalamectomy or anterior median eminence lesion can be divided into two groups as regards adrenal responsiveness *i.e.* response in acute experiments and response in chronic ones. In acute experiments there was some responsiveness of the adrenal cortex to stress and ACTH, whereas in chronic experiments the responsiveness was lost. Strong light or sound stimuli were ineffective in eliciting increased adrenocortical response. In these lesioned animals the adrenal gland was *normal* and not atrophic and there was *some basic secretion of 17-OHCS*, while in hypophysectomized animals the adrenal gland was *atrophic and 17-OHCS was at zero level*. Though the basal level of 17-OHCS was low in the lesioned animals, it was not at the zero level as was in hypophysectomized animals. This brings us to a question of either a basal secretion of ACTH in the lesioned animals or of two ACTH. One maintains the growth of the adrenal cortex and the other is activated during demanding situations through hypothalamus and median eminence.

**C. Photographic studies**—From the studies, encephalic penetration of light in the *Palaemon carcinus*, *Bufo melanostictus*, *Lates calcalifer*, guinea pigs and rabbits were noted. Light passed through openings, foramina, gaps, suture lines etc. The more transparent the structures, the more light passed as noted in *Palaemon carcinus*. This raised the question of direct effect of light on the hypothalamus, and specially the maturation of the hypothalamus partially by the effect of light and environment cannot be neglected. Environment had a tonic influence on the maturation of the hypothalamus not only regarding the pituitary-gonad axis but also the pituitary-adrenal axis. There was a difference in the steroid level in between new-born babies reared in light condition and in unhealthy environment (dark). Babies reared in light condition showed high 17-OHCS levels as compared to the other group. Light stimulus reached the hypothalamus not only through the eyes but also direct encephalic penetration occurred through the foramina, un-



united sutures and fontanelles. Fontanelles are important avenues in newborn babies through which direct encephalic penetration of light occurred to stimulate and mature the hypothalamus.

### DISCUSSION

The median eminence is important for the seasonal variation in toads, diurnal variation in guinea pigs, and diurnal variation in dogs with regard to 17-hydroxycorticosteroids secretion as after the lesion of the median eminence such variations are not found. Hokfelt and Luft (1959) found that in patients with suprasellar tumours distorting the hypothalamus, the normal diurnal variations in 17-OHCS in plasma, as well as the response of aldosterone excretion to salt restriction, may be absent.

Pellegrini *et al* (1958) have shown the importance of photostimulation in man as cofactor of regulation of various vegetative functions. "It appears likely that this effect takes place by means of diencephalic stimulations, especially on account of the different aspects which it shows in normal subjects and in subjects in whom the existence of a lesion or of a dysfunction of the diencephalon is either proved or probable." Mosinger (1958) reviewed the previous observation regarding the connexion of the optic nerves with the hypothalamus. Optico-hypothalamic connections and the importance of the hypothalamus with regard to the pituitary-gonad-axis and other functions have been reviewed previously.

In the *Bufo melanostictus* constant illumination gave rise to an increase in 17-OHCS content while darkness led to a fall. Fracture and scald were good stressing procedures and gave rise to increased 17-OHCS level. After hypophysectomy the responses were lost. Pituitary stalk section abolished the response to constant illumination but after fracture and scald some response, was noted. Median eminence lesion blocks the response to constant illumination and fracture but after scald there was some increased response. This might be due to the liberation of some chemicals at the site of scald and their action on the adenohypophysis. Audiogenic stimuli led to increase in the weight of the adrenal cortex (D'Amour and Shaklee, 1955) and there was an increase in the corticosteroid content (Duncan, 1957) though there was no change in the cholesterol content in a single test (Duncan, 1957; Anthony and Babcock, 1958). Fortier (1951) found eosinopaenia after sound stimulus. Biro *et al* (1959) found that "the sound stimulus evoked in part the usual change in the pituitary-adrenocortical system (characteristic to systemic stress) but in part inhibited: (1) the well known eosinopenic effect and (2) the decrease which should be brought about in any test apart from the effect of the sound stimulus by the conditions accompanying activity and seizures." Sackler *et al* (1959) reported that intense sound stimulation led to weight gain reduction and serious changes in endocrine weight and histological structure of the laboratory rat. Adrenal hyperplasia and significant changes in pituitary cell type were noted. In the present investigation auditory stimuli led to increase in plasma 17-OHCS content in the peripheral blood in guinea pigs and adrenal venous 17-OHCS output in dogs. Hypophysectomy, pituitary stalk-section, lesion of the anterior median emi-



nence and ventral hypothalamectomy blocked the response. de Groot and Harris (1950) performed remote control stimulation of the hypothalamus and pituitary gland in the conscious, unrestrained rabbits and from stimulation of the following areas positive response (in the form of 3rd hour maximum lymphopenia) was noted :—*Posterior region of the tuber cinereum, mammillary body and neurohypophysis*. This procedure stimulated the fibres of passage from the reticular formation to the hypothalamus and thus the increased response was noted.

Exposure to strong light showed a similar feature as was observed with sound stimuli in different experimental preparations. Fracture and burn led to increased adrenocortical response in guineapigs and dogs. Hypophysectomy blocked the response. With partial hypophysectomy (50%) in dogs increased response was noted after fracture, burn and ACTH. This showed the presence of adrenal responsiveness in such dogs. In acute experiments with ventral hypothalamectomy and lesion of the median eminence, increased adrenocortical response was noted after fracture, burn and ACTH in guineapigs and dogs. In chronic experiments the responses were meagre. After pituitary-stalk-section, fracture and burn led to increased adrenocortical response in guineapigs and dogs. In hypophysectomized guineapigs some increased adrenocortical response was noted but the response was of lesser magnitude in comparison to that noted in normal animals. In hypophysectomized dogs response after ACTH injection was of a very low order. After pituitary-stalk-section good response was noted after ACTH injection both in guineapigs and dogs. Hokfelt *et al.* (1959) found that "hypophysectomy in man resulted in the disappearance of non-conjugated cortisol and cortisone, as well as of the corresponding tetrahydro-derivatives. In agreement with this, a rapid and marked decrease in total 17-ketogenic steroids was recorded. Section of the pituitary stalk was found to produce an increase in the urinary output of these metabolites. As regards, 17-ketosteroids, changes parallel to those for 17-ketogenic steroids were observed. For aldosterone, an increase in the urinary excretion was found in all three cases." After optic nerve lesions there was very small increase in adrenocortical response in guinea-pigs after light stimulus, may be by a direct effect of light on the hypothalamus.

Roy (1959a) showed that stress response in the form of activation of the pituitary-adrenal-axis can be achieved after fractures and other forms of stress even in the absence of intact spinal cord. Roy (1959-b) found that there was a rise in adrenal venous 17-hydroxycorticosteroids output after fracture and burn injury applied to the denervated extremity. Fracture in a neurectomized limb with compression bandage applied did not show increase in 17-OHCS output. In midbrain-sectioned dogs, the increase in 17-OHCS output after fracture and burn was not much and this suggested a control of the adeno-hypophysis by the mid brain mediated through the hypothalamus. Roy (1959-b) also showed that fracture and burn trauma in pituitary stalk sectioned dogs gave rise to increase adrenal venous 17-OHCS output. Roy (1959c) observed the effects of different types of fractures, operations on bones and burn on the pituitary-adrenal axis of frontal leucotomized animals. Maximum adrenocortical activity



was noted. This was due to the fact that after frontal leucotomy, the hypothalamus was released of the inhibitory influences from the frontal cortex and as such, it worked in a more increased way, leading to increased ACTH secretion from the anterior pituitary, which acts on the adrenal cortex. The same view was already expressed by Lissak (1956) and Endoczi and Lissak (1953) using different types of stressors. In spinal transected guineapigs and dogs increased adrenocortical response after fracture and burn was noted in the present investigation but the magnitude of response was less than that observed in control animals—subjected to similar stress. Anderson and coworkers (1957) found an increase in urinary steroid excretion after laparotomy in dogs with transected spinal cord. Gordon (1950) found that severe burn of the denervated leg produced adrenal ascorbic acid depletion. Very severe scald to the leg caused some eosinopaenia in spinal transected dog (C7) (Hume, 1952). Hume (1958) mentioned that three days after cord section at C7, abdominal laparotomy still gave rise to the release of ACTH, although the response was slower to start than in the intact animal. This was due to the release of some substance in the injured tissues and its transport to the brain by the blood-stream. "It would appear that both nervous impulses and the supposed humoral factor act through excitation of nervous pathways which ultimately lead to release in the anterior median eminence, of an anterior pituitary stimulating substance." Hume and Egdahl (1959) observed no increase in adrenocortical secretion in response to a severe burn of the denervated leg but increased response after burn-injury to the denervated limb was seen (See Table I, p. 3) in some of the experiments. *e.g.*, Exp. No. 3 where the control value is 1.5 and 30' after, the value was 7.2. Exp. No. 5 shows 0.8 as control value and 2.7 at 90' response after ACTH or burn above site of section has not been presented. The dog in Exp. No. 8 had high control value *e.g.* 7.4 and the response after ACTH was only 11.8. Egdahl (1959) studied pituitary-adrenal response following trauma to the isolated leg of dogs. It is concluded that following severe burns or operative trauma, the adrenocortical stimulation *exclusively* depended on the peripheral nervous impulses and also there was no evidence for the production in the wounded area of a substance which stimulated the pituitary-adrenal-axis. By isolated-leg is meant that the leg is attached to the body only by one artery, one vein and one nerve. In such experiments, immobilization of the operated extremity was achieved by application of a plaster cast around the operated area and extended from the knee to the groin and passed around the body of the animal. External immobilization with plaster cannot completely immobilize the extremity and more so when the animal kept moving all the time. There is every possibility of kinking of the vessels with accentuation of the oedema and least absorption. The only form of perfect immobilization would have been by internal fixation of the transected femur with intra-medullary pin or plates and screws. "When, in addition, the thigh is transected, leaving the femur, femoral artery, nerve and vein, *the leg swells for about one or two weeks*"—(Markowitz *et al.*, 1959—*Experimental surgery*). The oedema retards absorption and more commonly there is thrombosis. Experimental work in our laboratory showed importance of both nerves and vessels in carrying the stress message to the brain. In Egdahl's (1959)



experiments performed 14 to 21 days after isolated leg preparation (nerve transected at original procedure), no response was obtained after the isolated, denervated leg was burned in the standard fashion. Similar experiments in our laboratory showed increased 17-OHCS output after burn injury. This was because of *nerve regeneration* and *vascular regeneration* as no step was taken either by Egdahl or myself to prevent the regenerations and regenerations are more quick in fracture haematoma (in our case haematoma formed at the site of bone sections).

The question arises: whether the burned or injured area produced chemicals which have got some influence on the pituitary-adrenal-axis. This was dealt with by Roy (1959-b), who showed that the burned or injured area produced some chemical and thus the venous blood collected from such extremities increased the 17-OHCS output in the test dogs when injected into the carotid artery. Subcutaneous pockets were made in guinea pigs. After burn to the superficial skin, fluids accumulated in the subjacent pockets. When this fluid was injected into normal guinea-pigs, there was increase in 17-OHCS content in the blood. One of the most important constituents of the fluid is histamine (Roy, 1959d). Moreover Moon in 1942 stated, "In previous discussions the results of many workers were cited indicating that the prolonged stimulation of peripheral nerves will not cause manifestations of shock; also that complete anaesthesia prevented sensory impulses, originating in the periphery from reaching the brain, and that shock was *not* prevented by severing all nerve paths between the traumatized limbs and the brain. It has been shown that roentgen irradiation of the abdomen caused the complete syndrome of shock. This type of injury is entirely painless, hence it would not cause noxious nerve impulses. These observations weigh against the theory that nociceptive nerve impulses, are important factors in the dynamics of shock." Markowitz *et al.* (1959) mention regarding the shock in burn that "Although it is reasonable to accept these experiments at their face value, since the shock is easily cured by transfusion of plasma, it was difficult to explain why the severely shocked animal, in spite of transfusion, so often died. Again, a toxin was postulated." Cannon (1923) postulated that there was absorption of some sort of undefined toxic material from large soft tissue wounds into the circulation and this gave rise to the occurrence of profound shock. Underhill *et al.* (1930) stated that methylene blue and phenolsulphonthalein were absorbed slowly from the injured tissues. Cameron *et al.* (1943) found that there was significant systemic absorption of tannic acid after application on the burned area and occasionally it was to a toxic level. Wise *et al.* (1958) measured the rate of absorption of ( $I^{131}$ ) and iodinated ( $I^{131}$ ) human serum albumin from large soft tissue wounds in the hind legs of Texas Angora goats. They found that both of these substances were detectable in the blood, one hour after application to the wound.

In the isolated-pituitary experiments, the only parts left in the calvarium were the pituitary, cerebellum, pons, midbrain and medulla. These animals had very high 17-OHCS output and after ACTH and the stress of fracture and burn, 17-OHCS output was higher. The results indicate that as the inhibitory influences from higher centres are removed, the



pituitary can activate in a more pronounced way. A similar opinion has been expressed by Hume and Egdahl (1959). The pituitary has got no nervous connection with the brain, but only systemic vascular connection and its control is by :—(a) neuro-secretion of the pons, medulla, cerebellum and midbrain circulating through the systemic circulation ; and (b) histamine released at the injured site and acting on the isolated pituitary.

The pituitary gland is very labile in such conditions and any chemical alteration or pH change in the blood will lead to increased ACTH discharge. Sayer's hypothesis may also be considered in such contexts.

The discussions about the adrenal changes and photographic studies have been already mentioned as also changes in the neurosecretion. Neuro-secretory material has a stimulating action on the anterior pituitary ACTH discharge during environmental stimuli and stress. Increased activity in the pituicytes was noted during stress. Leveque and Small (1959) state that the pituicyte is implicated in either the release mechanism or the separation of the hormone from a carrier substance. A similar opinion was derived from the observations in the present investigations. Separation of the hormone from the carrier substance is possible in the median eminence by chemical processes and the hormone enters into the hypophysiportal vessels to be migrated into the adenohypophysis where it stimulates ACTH secretion. Stutinsky (1958) found large Herring's bodies in hypophysectomized or post-hypophysectomized animals and a great number of cells acquired a basophilous character.

### CONCLUSIONS

Environmental stimuli in the form of light and sound exert a tonic influence on the hypothalamus regarding the hypothalamo-pituitary-adrenocortical activity. Light also helps in the maturation of the hypothalamus for the activity of the same axis. Light stimuli not only influence the hypothalamus through the optic nerves but also a direct encephalic penetration of light occurs in order to mature and stimulate the hypothalamus in some species. For the proper activation of the light and sound stimuli, the hypothalamus, the median eminence, the hypophysiportal vessels and the hypophysis must remain intact.

The neurosecretory system, the pituitary and the adrenal gland were studied after light and sound stimuli and also after fracture and burn in different experimental preparations.

The importance of the spinal pathways, frontal lobe of the brain, other areas of the brain, the hypothalamus, the median eminence, the hypophysiportal vessels in different stress situations with respect to the pituitary-adrenocortical activity has been mentioned.

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## CHAPTER—13

### THE RELEASE-MECHANISMS OF THE HORMONES OF THE ANTERIOR PITUITARY (1973)

McCann and Porter (1969), Meites (1970), and McCann (1971) made scholarly reviews on hypothalamic pituitary stimulating and inhibiting hormones and the mechanism of their action. Corticotropin-releasing factor (CRF), thyrotropin-releasing factor (TRF), luteinizing hormone-releasing factor (LRF), follicle stimulating hormone-releasing factor (FRF), prolactin-inhibiting factor (PIF), growth hormone-releasing factor (GRF), growth hormone-inhibiting factor (GIF), and melanocyte stimulating hormone-releasing and inhibiting factors (MRF and MIF) have been reviewed by McCann and Porter (1969). Methods for the assay of releasing factors have been mentioned by them also both in *in vivo* and *in vitro* experiments. Purification and separation of the releasing factors have been achieved by several chromatographic procedures.

The Doctoral thesis of Roy (1953/1954) embodied the works on the role of adrenal cortex and anterior pituitary in burns (accidental and experimental) and this was published in Calcutta Medical Journal in 1957. The suprarenal blood, after blocking the tributaries of the lumboadrenal vein was collected from the left side of an etherised and heparinized dog. The right adrenal was taken up for estimation of ascorbic acid and corticosteroid. The serum potassium was also noted from the peripheral vein prior to the experiment. The left adrenal gland was taken up for estimation of ascorbic acid and corticosteroid after the experiment was over.

Page 132 contains the details of the experiment number 15 (dated the 14th August, 1953). This experiment was performed on a male dog, 5½ kg. in weight. 3 c.c. of 1% potassium chloride was injected into the carotid artery and intracranial venous effluent was collected from jugular vein. 1 c.c. plasma from this blood was injected into the adrenal artery and the venous effluent was collected. There was a rise in the adrenal venous plasma corticosteroid content after such injection. Page 134 contains the details of the experiment number 21 (dated the 21st August, 1953) on a burned dog. There was a very small rise in adrenal venous plasma corticosteroid content, when similar procedures were adopted as in experiment number 15 by injecting 3 c.c. of 1% potassium chloride into the carotid artery and intracranial venous effluent was collected from the jugular vein. 1 c.c. plasma from this blood was injected into the adrenal artery and the venous effluent was collected for estimation of plasma corticosteroid content. The pituitary was damaged in the burned dog. Page 136 contains the summary of the results (Fig. No. 1). Page 137 contains two figures (Fig. Nos. 2 and 3) showing the results in a normal dog and in a burned dog. Page 140 contains figures demonstrating the adrenal ascorbic acid staining reactions in the control and the experimental guinea pigs (Fig. Nos. 4 and 5).



One of the conclusions that emerged from the clinical and experimental work was that "in burns there is *potassaemic hypercorticism* and as the level of potassium comes down there is a condition of *potassaemic eucorticism*." This was demonstrated in page 151 (Fig. Nos. 6 and 7).

That the hypophyseal portal blood contains releasing factor activity was first demonstrated by Porter and his associates (1956, 1959 and 1963). The pituitary region of the dog was exposed through temporal route. The pituitary was removed by suction and the hypophyseal portal blood was collected and assayed for CRF activity.

The method of Worthington (1966) for collection of hypophyseal portal vessel blood in rats included (1) reflection of lower jaw and tongue, (2) removal of the soft palate, (3) trephining of the basisphenoid bone and exposure and division of the stalk. The plasma was extracted and assayed for LRF activity.

Porter and Smith (1967) and Porter *et al.* (1971) described a method for collecting portal vessel blood in the rat. This method has several advantages :—

(1) Collection of stalk-blood may be done for 4-6 hours, (2) This blood is uncontaminated with extraneous fluids and (3) It comes from tissues unexposed to the desiccating action of air. The approach is through a parapharyngeal route. The pars distalis, pituitary stalk and postchiasmatic portion of the median eminence are exposed. Reflection of the dural covering of the pars distalis is done. The pituitary stalk is divided and the pituitary is removed. A polyethylene cannula is placed over the stalk. The cannula is fixed firmly against the diaphragma sellae and thus there is a liquid-tight seal. The cannula is fixed over the stalk by the help of a micromanipulator.

#### *Action of the releasing factor*

Douglas and Poisner (1964a) studied the mechanism of release of vasopressin from the posterior pituitary gland by *in vitro* method. The rate of release was greatly accelerated when potassium concentration was increased or after repeated electrical stimuli applied to the pituitary stalk. Calcium was essential for the response. Secretory response to excess potassium was strongly inhibited by magnesium. They said, "Attention is drawn to the similarities in the process of '*Stimulus-secretion coupling*' in neurosecretory cells, chromaffin cells and neurones and it is suggested that a common calcium—dependent mechanism may be responsible for release of their various products". Douglas and Poisner (1964b) studied calcium movement in the neurohypophysis of the rat and its relation to the release of vasopressin. Excess potassium (56 mM) greatly increased the rate of release of vasopressin by rat's neurohypophysis incubated in Locke's solution *in vitro*. The excess potassium caused a five-fold increase in the rate of  $^{45}\text{Ca}$  uptake. In stimulus—secretion coupling the impulse is propagated along the hypothalamo-hypophyseal tract and there is depolarization of the neurosecretory terminals with increased uptake of calcium. Thus there is extrusion of posterior pituitary-hormones from the neurosecretory terminals.



By increasing the concentration of  $K^+$ , augmented release from the rat pituitary gland was observed for LH by Samli and Geschwind (1968) and by Wakabayashi *et al.* (1968), for FSH by Wakabayashi *et al.* (1969) and Jutisz and de la Llosa (1970), for TSH by Vale and Guillemin (1967), for GH by MacLeod and Fontham (1970), for ACTH by Kraicer *et al.* (1969). When releasing factor and high  $K^+$  are added together in the medium, there is additive release of the hormone.

With high  $K^+$  and releasing factor (RF), the release is blocked by removal of  $Ca^{++}$ . For the blocking of RF-induced release, residual  $Ca^{++}$  is to be removed with ethylenediamine tetra-acetic acid (EDTA). Wakabayashi *et al.* (1969) noted blocking of FSH and LH secretion due to high  $K^+$  or RF by increased  $Mg^{++}$ .

Releasing factors depolarize cell membranes and there is increased  $Ca^{++}$  uptake which starts the process for release of the hormone. If this fact is true, then depolarization can be observed from pituitary cells by direct recording in response to hypothalamic extract. Ashworth *et al.* (1968) noted a long-lasting (1-2 min.) depolarization with extracellular microelectrodes in the anterior pituitary and this begins within 30 seconds after I.V. injection of hypothalamic extract to anaesthetized rats. Injection of cerebral cortical extract showed a smaller depolarization. No depolarization was observed when recording was taken from the pituitary stalk or basal hypothalamus. This proved that the negativity of the anterior lobe was not due to depolarization in tissues nearby. Milligan and Kraicer (1970) noted depolarization and even reversal of polarity of some cells with high  $K^+$  *in vitro*. They also noted good amount of variability in the resting potential of pituitary cells in the same conditions.

Roy (1962) studied the importance of zinc in the release of ACTH in the fish, guinea-pig and dog.

Female rat pituitaries *in vitro* cannot be stimulated to secrete prolactin by increased potassium ion concentration.

### Ultrastructural studies :

Emiocytosis is the process by which insulin from beta cells of the islets of Langerhans is released due to the movement of secretory granules to the cell-surface. The granules are encased in membranous sacs. The sacs fuse with the plasma membrane. Then they rupture and there is liberation of the granules into the extracellular space. Lacy and co-workers (1968) proposed a new hypothesis of insulin secretion. A microtubular-microfilamentous system interlinks the granules to the plasma membrane. Stimulation with glucose leads to contraction of this system resulting in fusion of the sacs encasing the granules with the plasma membrane and there is ejection of granules into the extracellular space. The contractile microfilaments may be of actomyosin-like material. Howell and Lacy (1971) gave a similar suggestion for insulin release.

Electromicroscopic studies were made on the somatotrops after GRF administration by Clementi *et al.* (1967), Ashby *et al.* (1967), Coates *et al.* (1968), de Virgiliis *et al.* (1968), Couch *et al.* (1969) and Coates *et al.* (1970).



de Virgillis *et al.* (1968) found evidences of increased protein biosynthetic activity. Coates *et al.* (1968, 1970) found the granule count to increase within 5 minutes, and it returned to normal by 15 minutes. The count significantly diminished by 30 minutes. Bioassays of GH, on the other halves of the same pituitaries correlated well with the changes in the counts of the granules. Increased protein synthesis was evidenced by the very prominent rough endoplasmic reticulum and the Golgi complex. Similar changes were noted also for gonadotrops. So, the hypophysiotropic hormone increases the synthesis and release of the GH. Smith and Dhariwal (1970) noted inhibitory changes for synthesis and release of prolactin after administration of PIF.

Farquhar (1971) proposed events in the secretory process of mammothrophs in the anterior pituitary of the rat. Synthesis of MTH occurs on ribosomes. It is segregated and transported by the rough ER. The Golgi complex concentrates into granules. Mature secretory granule is formed by aggregation of small granules arising within the inner Golgi cisterna. When active secretion takes place, there is fusion of the mature secretory granule with the cell membrane and it is discharged into the perivascular space by exocytosis. When there is cessation or slowing of the secretion, crinophagy occurs. By this process the granules move toward and fuse with the pre-existing lysosomes.

#### RELEASE OF ANTERIOR PITUITARY HORMONES AND THE EFFECT OF INHIBITORS OF PROTEIN AND RNA SYNTHESIS

Actinomycin D is an inhibitor of RNA synthesis. Puromycin or cycloheximide inhibits protein biosynthesis. Release of pituitary hormones other than growth hormone is not affected by inhibitors of proteins and RNA synthesis. Jutisz *et al.* (1970) postulated that "the stimulatory effects of hypothalamic factors on the release of pituitary hormones do not require synthesis of an intermediate protein or RNA. On the other hand, the inhibition of the effects of TSHRF by thyroid hormones may be mediated through one or several substances, the synthesis of which can be inhibited by antibiotics."

#### RELEASING FACTORS AND ADENYL CYCLASE SYSTEM (SUTHERLAND AND ROBINSON, 1966 and ROBINSON *et al.*, 1968)

Adenyl cyclase is located in the pituitary cell membrane. This enzyme catalyzes the formation of cyclic AMP (3', 5'-adenosine monophosphate) from ATP. The enzyme phosphodiesterase catalyzes the conversion of cAMP to AMP. Methylxanthines *e.g.* caffeine and theophylline inhibits phosphodiesterase activity.

Releasing factors act on adenyl cyclase in the pituitary cell membrane. cAMP increases in the cell and in some way there is release of the hormone. McCann (1971) put forward a hypothesis in which releasing factors are thought to combine with specific receptors on the surface of



the cell. There is activation of adenylyl cyclase with increased intracellular level of cAMP. There is increased permeability of the cells to  $\text{Ca}^{++}$  and there is extrusion of the granule. Aminophylline and cAMP stimulates release of some pituitary hormones. Jutisz *et al.* (1970) thought that for the release of at least FSH, LH, TSH, the following features occur :— (1) The intermediate between releasing factor and release is probably cAMP. (2) For release of the hormone to occur, a high  $\text{K}^{+}$  concentration in the external medium acts as a non-specific stimulus. (3) Presence of  $\text{Ca}^{++}$  in the external medium is necessary for the action of RF and increased  $\text{K}^{+}$  on the cell to occur.

Increased  $\text{K}^{+}$  leads to increase in the level of cAMP (Rasmussen and Tenenhouse, 1968). Zor *et al.* (1969) found that increase in  $\text{K}^{+}$  will not increase cAMP in the pituitary tissue.

McCann (1971) speculated on the action of inhibitors of release *e.g.* PIF, GIF, and melanophore stimulating hormone inhibiting factor (MSHIF). These inhibitors reduce adenylyl cyclase activity and there is reduction in cAMP levels. There is reduced membrane permeability and hyperpolarisation. Diminished intracellular  $\text{Ca}^{++}$  will reduce the release of the hormone.

McCann (1971) further states, "Target gland hormones are visualized as feeding back at the pituitary level to produce inhibitory proteins or peptides which alter basal and RF—stimulated release of pituitary hormones."

### *How $\text{Ca}^{++}$ helps ?*

Geschwind (1970, 1971) put forward several theories in order to explain the role of  $\text{Ca}^{++}$  in the release process. The granules in the cell are linked to the plasma membrane by actomyosin-like microfilaments.  $\text{Ca}^{++}$  promotes contraction of these microfilaments and thereby facilitates release of the granules. According to Rasmussen and Tenenhouse (1968), a  $\text{Ca}^{++}$ —ATP complex was pictured.  $\text{Ca}^{++}$  acts in the cytosol and adenylyl cyclase acts upon ATP to produce cAMP.

Mechanism of the release of pituitary hormones by the action of  $\text{K}^{+}$  and releasing factors (RF's) has been illustrated in figure number 8.

### CONCLUSION

Stimulant action of potassium on the anterior pituitary and the adrenal cortex was shown by Roy (1953/1954). The indices used were cortical hormone output (in adrenal vein), adrenal corticosteroid content and adrenal ascorbic acid depletion. No explanation regarding the mechanism of the stimulant action of potassium in the release process of pituitary hormone (ACTH) could be offered then ; but at present the mechanisms of action of  $\text{K}^{+}$  and the releasing factors have been well documented by many authors with the help of *in vitro* experiments, ultra-structural studies and others.





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## Chapter 13

- a) Experiments were carried out in normal as well as in burned dogs.
- b) The serum Potassium was high in the burned dogs.
- c) The basic adrenal cortical output in the burned dogs was high.
- d) The adrenal ascorbic acid and the corticosteroid was low in the burned dogs. They were further lowered by the experimental conditions.
- e) 1 per cent Potassium chloride could stimulate the adrenal cortex in a better way in comparison with 3 per cent Potassium chloride.
- f) Potassium Acetate and Bicarbonate were better stimulants than Potassium chloride.
- g) Potassium Nitrate had a stimulating action but inferior to Potassium Acetate and Bicarbonate.
- h) Vitamin C in smaller dose had a stimulating action in burned dog.
- i) A.C.T.H. had a greater stimulating action in the burned dog than in the normal one.
- j) Pituitary could not be directly stimulated with Potassium chloride but indirect means had to be adopted by injecting the solution into the carotid artery and collecting the effluent from the Jugular vein. In the ~~normal~~ <sup>burned</sup> dog the response was not as good as in the normal one (The Pituitary was damaged in the burned dog).

Fig. 1. Summary of the results.



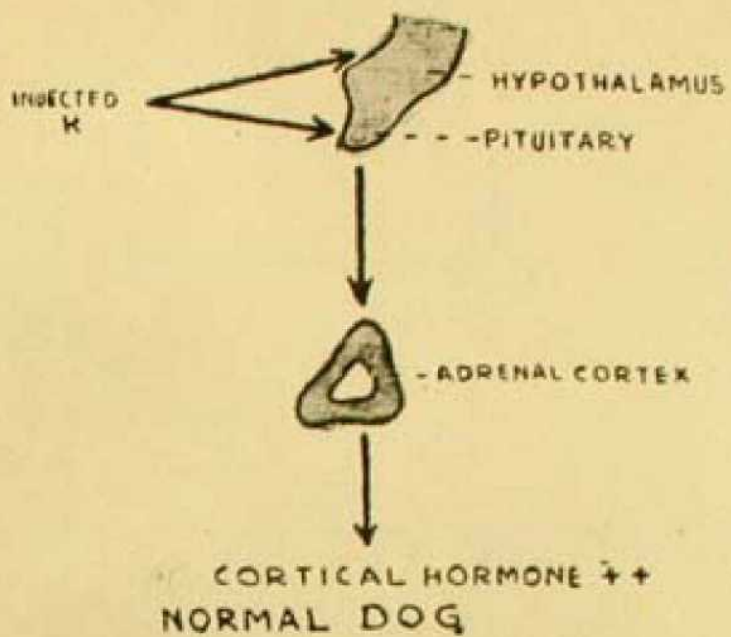


Fig. 2

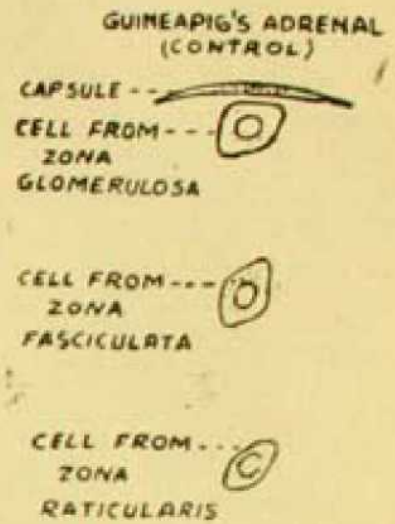


Fig. 4

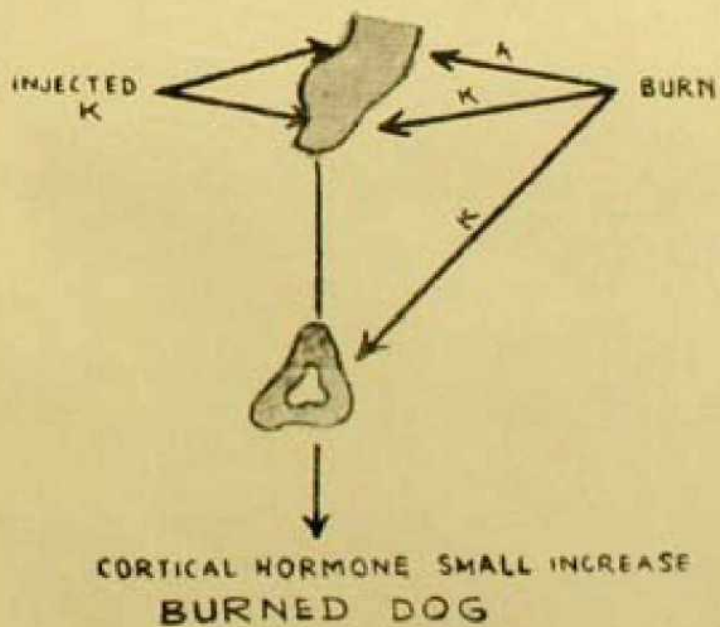


Fig. 3

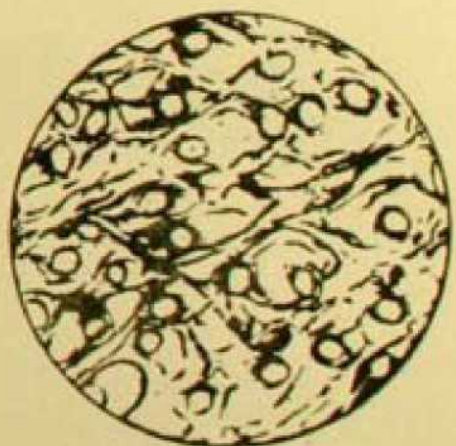


Fig. 5

Figs. 2 & 3. Result of experiment Nos. 15 & 21.

Figs. 4 & 5. Adrenal ascorbic acid staining reactions in normal and experimental guinea pigs.



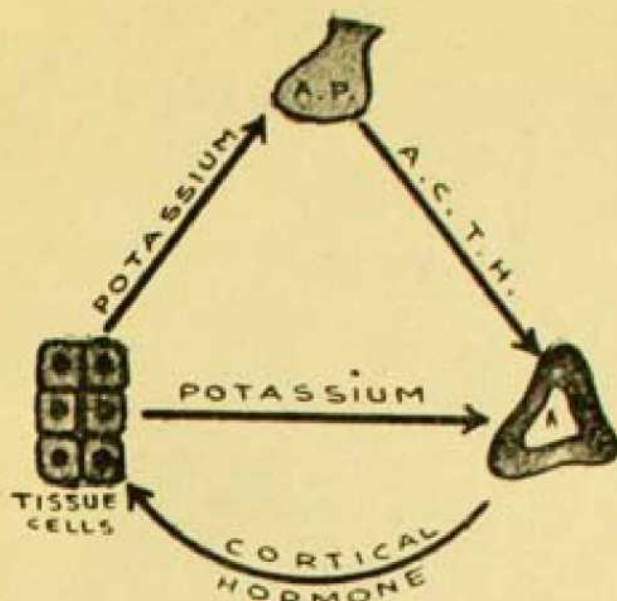


Fig. 6

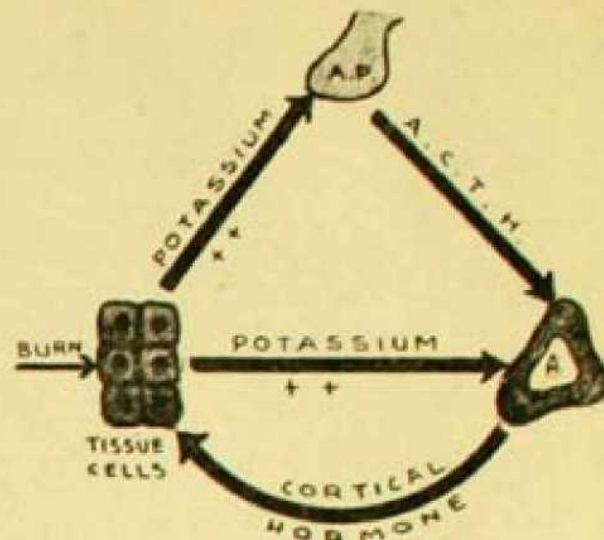


Fig. 7

Figs. 6 & 7. Potassaemic hypercorticism in burns.

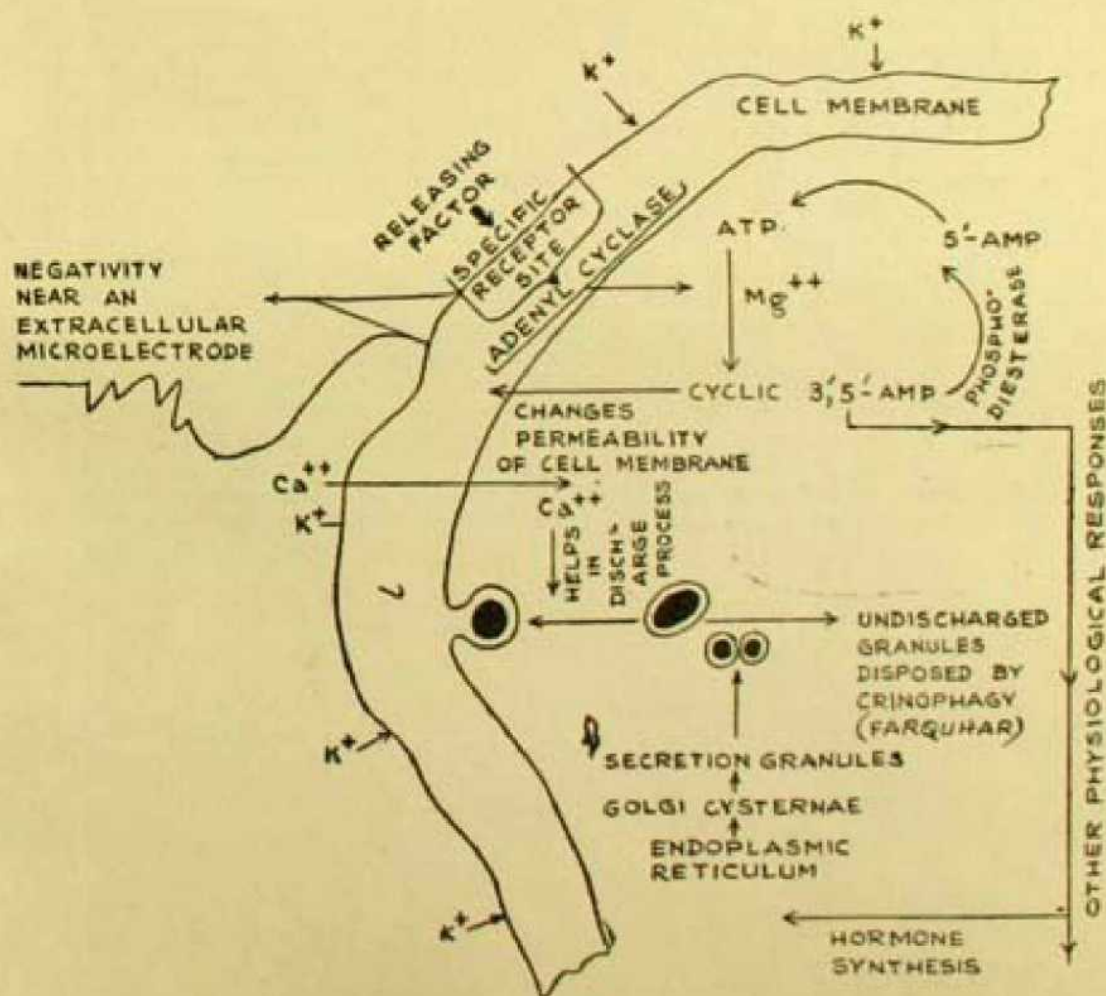


Fig. 8 - MECHANISM OF THE RELEASE OF PITUITARY HORMONES BY THE ACTION OF  $K^+$  AND RELEASING FACTORS.

Fig. 8. Mechanism of the release of pituitary hormones by the action of  $K^+$  and releasing factors.





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## CHAPTER 14

### STRESS AND INVERTEBRATE NEUROSECRETION WITH RECENT STUDIES ON CAUDAL NEUROSECRETORY SYSTEM

1974

#### INTRODUCTION

##### *Concept of Neurosecretion :*

Knowles and Bern (1966) discussed the function of neurosecretion in endocrine regulation. Secretory cycle as noted in gland cells was demonstrated in certain neurones of the central nervous system by specific staining reactions. This was the first phase in the development of the neurosecretory concept. In the second phase hormonogenesis in those neurones was identified, e.g., antidiuretic hormone in vertebrates, crustacean chromactivating hormone, prothoracotropic brain hormone in insects. Neurohaemal concept was developed by Knowles and Carlisle (1956). This concept includes hormonogenesis in neurosecretory cells and discharge of the neurosecretory product in blood vessels of a neurohaemal organ. Neurosecretory innervation of endocrine cells (e.g., vertebrate pars intermedia, pars distalis of some teleosts and corpora allata of some insects and others) has been found out with electron microscopic studies. Vesicles and granules resembling those noted in cholinergic and adrenergic nerve endings have been observed in the median eminence of the amniote. Special secretory cells in the ependymal floor of the infundibular recess of the dogfish *Scyliorhinus stellaris* have been noted by Knowles (1971). These cells communicate with the cerebrospinal fluid but secrete into the hypophyseal-portal system and they might contain gonadotrophin-releasing factors. Rodriguez (1971) noted that inhibitory control of the pars intermedia in amphibians and probably in mammals was effective mainly or exclusively by neuroglandular contacts. In the lizard the control was thought to be neurovascular. Less significant neurovascular control of nonreptilian pars intermedia should not be disregarded at present. Electron microscopic studies of the neurosecretory innervation of pituitary intermedia cells of *Scyliorhinus* were carried out by Knowles (1965). Two types of innervation were noted. Peptidergic fibres or type A fibres made direct synaptoid contacts with the intrinsic intermedia cells at their synthetic poles. Aminergic or type B fibres made contacts similarly at the release poles. Knowles, Weatherhead and Martin (1970) noted more complex innervation of intermedia cells of the dogfish. Knowles (1965) proposed two main types of neurosecretory systems. Type A or peptidergic fibres release peptide substances and there are Type B or aminergic fibres. Pituitary is innervated mainly by Type B fibres. Vollrath (1967) summarised different forms



of neurosecretory innervation of pituitary cells. In the teleost fish *Tinca* single or double basement membrane separates the neurosecretory terminals from the pituitary cells. In the *eel* the separation is greater. So the neurosecretory hormones will act on the pituitary cells by diffusion through wide area and thus only few neurosecretory fibres can control many pituitary cells. However, in the *Hippocampus* or *Scyliorhinus* direct innervation of the pituitary cells has been noted. This type of innervation necessitates plenty neurosecretory terminals in order to influence individual cells. In higher forms (mammals) there are terminations of neurosecretory fibres in the median eminence from where the hormones are carried to the cells of the pituitary by hypophyseal portal vessels.

Bern (1971) compared the innervation of the pituitary of two euryhaline teleost fishes, *Gillichthys mirabilis* and *Tilapia mossambica* and he studied the origin and nature of Type B fibres. Knowles Type A fibres contain typical elementary neurosecretory granules (ENG) and the Type B fibres contain large dense-cored or granulated vesicles (LGV). Presynaptic axonal membrane modifications were noted in *Gillichthys* but were absent in *Tilapia*. Bern concluded that (Type B) LGV containing fibres innervating the different adenohypophyseal cells in these two fishes are monoaminergic. The dense core contained an active catecholamine and a carrier protein. According to him these Type B fibres are same as adrenergic fibres that are found to contact neurohaemally in the outer layer of the median eminence of lower actinopterygians, lungfish and tetrapods. Some of the LGV—containing neurones of the Nucleus Lateralis Tuberis (NLT) of teleosts may be equivalent to the infundibular nucleus of amphibians and birds and the arcuate nucleus of mammals which contain catecholamines and project to the outer layer of the median eminence and thereby control adenohypophysial function. This strongly supports the theory of neurosecretory innervation.

Knowles and Bern (1966) state that though there is diversity in the form and function of neurosecretory neurones including biochemistry of the hormones, yet there is one feature common to them. This is their direct or indirect participation in endocrine control and thus they form all or part of an endocrine organ. They further state, "Morphologically, neurosecretory neurones combine neuronal and glandular characteristics. Physiologically also they provide an essential link between the nervous and endocrine systems. As such they provide the final common pathway for neuroendocrine regulation."

Scharrer and Weitzman (1970) discussed the current problems in invertebrate neurosecretion. Type A neurosecretory neurones are peptidergic and aldehyde fuchsin positive. Polypeptides elaborated by them are rich in cysteine or cystine. The second type of neurosecretory neurones is known as Type B neurones. These are aminergic and aldehyde fuchsin-negative and contain little or no cystine. They contain biogenic amines. The neuronal properties of invertebrate neurosecretory cells are there. They have got the capacity for generating and conducting impulses. Slow conduction velocity in the action potentials has been noted in invertebrate neurosecretory neurones. These neurones in the abdominal ganglia of aquatic gastropod *Aplysia* can act like pacemaker



cells. Sensory antidromic conduction from osphradium (a receptor organ in the mantle cavity) by the branchial nerve can increase autogenic activity of these neurosecretory cells (Jahan-Parwar *et al.* 1969). Scharrer and Weitzman further state that photo-neuro-endocrine pathways could be detected in insects. Calcium is necessary in the release-phenomenon of neurosecretory materials in invertebrates. Cyclic AMP has its definite role in mediation of hormone action in effector cells.

Neurosecretion in invertebrates has been studied by many for a long time ; but some groups have been investigated in more details than others. Gabe (1966) has discussed different factors for this state of affair :

- (1) Ease or difficulty in availability of a particular material than other,
- (2) Easy or difficult-maintenance of that material in the laboratory, either in optimal or in different experimental conditions,
- (3) Handling the material for surgical purposes and development of difficult surgical techniques which are not very typical as one interested in vertebrate neuroendocrinology may not find it suitable for the invertebrate group. Very few investigators include their studies both in vertebrate and invertebrate neurosecretion.

Whatever may be the difficulties, one common finding that has emerged out of prolonged research works of different investigators, is that there are :

- (1) neurosecretory cells
- (2) the axonal paths for migration, and
- (3) the end station where the material is collected to be discharged when required. This material then acts on different endocrine organs in animals possessing them.

Different review papers and books are there wherein anatomy and physiology about neurosecretory system have been well-discussed (Heller and Clark, 1962 ; Von Euler and Heller, 1963 ; and Rockstein, 1964 ; Bullock and Horridge, 1965 ; Gabe, 1966 ; Martini and Ganong, 1967 ; Bargmann and Scharrer, 1970 ; Heller and Lederis, 1971 and others).

In the preface of Gabe's book on neurosecretion the late Professor Bertil Hanstrom said, "It is easy to predict that this work will be the Bible of neurosecretion for many years to come and, indeed, I believe that in the future no single scientist will ever be able to deal with the anatomy and physiology of the neurosecretory organs in the whole animal kingdom in one and the same volume as Dr. Gabe has done."

Different functions have been ascribed to the neurosecretory materials, and they have been discussed in details by Bern and Hagadorn (1965), Bern and Knowles (1966), Hagadorn (1966), Gabe (1966) and others. Changes in neurosecretory system of invertebrates in response to stress have not



been thoroughly investigated and references are very few. So, in this present investigation my own observations will be put forth along with those of others. The other functions of the neurosecretory system will be mentioned briefly.

## I. NEUROSECRETION IN INVERTEBRATES

Neurosecretory cells have been described in *Hydra*. Lentz (1968) found that the membrane—bounded granules of moderate density seemed to originate within Golgi cisternae of the cells. Neurosecretory cells have also been noted by Lentz (1968) in the planarian. Large membrane—bounded granules of varying electron density have been noted in the cells. At the periphery of the Golgi cisternae there is electron-dense material. It was thought that the small vesicles with same electron density nearby were derived from the periphery of the Golgi cisternae.

### PLATYHELMINTHES

The peripheral arrangement of cells and the inner core of neuropiles (fibres of passage and terminations) in the brain of flatworms, is a complex type of arrangement as noted in higher invertebrates.

In the central nervous system of higher invertebrates unipolar type of nerve cells are found. The turbellarian central nervous system contains unipolar, bipolar and multipolar nerve cells. From the works of Hadenfeldt (1929) (Fig. 1) and Turner (1946) different types of nerve cells have been known to occur. They have been designated as Types A, B, C, D, E, F, G, H, J. Uniform round granules are found in the cell-J and this type is possibly a neurosecretory one.

In the postero-ventral part of the brain of *Polycelis nigra* Lender and Klein (1961) noted angular neurones. These are paraldehyde-fuchsin-positive neurosecretory cells. After amputation of the posterior segment, the number of the neurosecretory cells increases during the first few days. The angular cells become rounded. The indistinct nucleus and nucleolus become more distinct. The cytoplasm is more prominent. Axonal migration of the neurosecretory material was not observed but they noted paraldehyde-fuchsin-positive tracts during posterior regeneration. Induction of regeneration of excised eyespots from a distance, is caused by the cerebral ganglion.

### NEMERTEA

The cerebral ganglia have four lobes, a dorsal pair and a ventral pair (Fig. 2). The dorsal commissure connects the two dorsal ganglia passing dorsal to the rhynchodaeum; the ventral commissure connects the ventral ganglia and it is situated ventral to the rhynchodaeum. The ganglia and the commissures form a collar of nerve surrounding the proboscis apparatus.

The neuroglandular cerebral organ (Fig. 3) has relationship with the cerebral ganglion (Scharrer, 1941). Berta Scharrer found identical appearance of the ganglion cells of the cerebral ganglia and the cerebral organ.



True neurosecretory cells in the cerebral ganglia of Lineidae were observed by Lechenault (1962). There is possible relationship between the activity of the cerebral ganglion and spawning (Gontcharoff and Lechenault, 1958). Division of the *Lineus* into two, causes the ovules of the posterior portion to mature ; but immaturity of the ovules in the anterior half (with the brain) is noted.

### NEMATODA

The nervous system of *Ascaris lumbricoides* consists of a circumenteric ring. It surrounds the pharynx. Ganglia are connected with the ring. From the ganglia longitudinal cords proceed in both anterior and posterior directions. The ganglia are named as paired lateral and ventral ganglia, a small dorsal ganglion, a pair of subdorsal ganglia, a pair of sublateral ganglia, and a pair of postventral ganglia. Commissures connect the ganglia with the nerve ring or connect the ganglia themselves. The cerebral ganglia are represented by the lateral ganglia. Regarding number, position, shape of the nerve cells and course of the fibres, there is constancy. Gersch (1957) and Gersch and Scheffel (1958) described neurosecretory cells in the nerve ganglia of *Ascaris lumbricoides*. Neurosecretory cell has been found by them in the ganglia of the large lateral papillary nerves. Axonal migrations have also been noted more in the commissures than in the papillary nerve. End stations have not been described.

### ANNELIDA : POLYCHAETA

In polychaete annelids neurosecretory cells have been described and the physiology of neurosecretion in this group has been well described (Fig. 4). Berta Scharrer (1936, 1937) described four different types of neurosecretory cells in Nereidae. *Cells a* had homogeneous and acidophil cytoplasm. Fusiform *cells b* had vacuolated cytoplasm and strongly fuchsinophilic large granules filled the vacuoles. Rounded *cells c* had fine granules in the cytoplasm and large vacuoles in it. *Cells d* had nucleus pressed to the cell wall. The cytoplasm was reduced. There was a large vacuole with fuchsinophilic droplets or pools in it. Schaefer (1939), Harms (1948), Bobin and Durchon (1953), Hauenschild (1959) and Hauenschild and Fischer (1962) described neurosecretory cells. Herlant-Meewis and van Damme (1962) described neurosecretory cells in *Nereis diversicolor*.

Clark (1959) described types A, B, C, cells in nephtydae. Gabe (1966) states that in certain stages of the developmental cycle, neurosecretory substance passes along axons into the integument and collects there in polychaete annelids.

### CEREBROVASCULAR COMPLEX

Transport of neurosecretion towards blood vessels has been described by Bobin and Durchon (1952).

Clark (1959) described the cerebrovascular complex as follows. In *Nephtys* a coneshaped structure is formed by a group of axons coming from



the central neuropil. The base of the cone is directed ventrally. Secretion of B type cell in nucleus W passes to the base of the brain along the axons. There is specialization of the membranes surrounding the brain at this junction and the plexiform dorsal blood-vessel is in close contact with the ganglion. Clark suggested this area to be a primitive neurohaemal organ which may be compared to the sinus gland in crustaceans and the corpus cardiacum in insects. Migration of neurosecretory material could, however, proceed along circumoesophageal connectives.

The cerebral ganglia in polychaete annelids control maturation of gonocytes, epitoky and posterior regeneration. A factor inhibiting spermiogenesis is elaborated by the cerebral ganglion (Durchon, 1951, 1952). This state of affair is not found to occur in females. But in *Platynereis dumerilii* the cerebral ganglia have an inhibitory effect on development of oocytes and spermatozooids (Hauenschild, 1956, 1959; Hauenschild and Fischer, 1962). Hauenschild (1956) described the active principle as "juvenile hormone."

Regarding posterior regeneration Harms (1948) proved in *Lycastis* that there was inhibition of regeneration after removal of prostomium and regenerative process started after implantation of a prostomium. This has been proved to be true by Clark and Clark (1959). Clark and Bonney (1960), Clark and Evans (1961) and Clark, Clark and Ruston (1962). Clark, Clark and Ruston (1962) concluded from their transplantation experiments with reference to regeneration in polychaetes that the ganglion did not contain the "regeneration hormones" effectively until the second or the third day after amputation of posterior segments. By the 4th/5th day the hormones are not present in the ganglia. Regeneration already being started, the removal of the ganglion at this time cannot stop the subsequent segment proliferation taking place. They further suggested that there was possibility of three sets of hormones in relation to regeneration. Cerebral hormone starting segment proliferation is known to them. The hormones control the rate of segment proliferation. "Finally, the prompt response of the neurosecretory system to wounding, the sudden increase in the number of blood cells and the important events taking place at the wound during the first three days, all suggest that other cerebral hormones may be concerned with the early stages of regeneration." Details of changes in the neurosecretory cells were presented by them in *Nephtys*. During the first six hours after amputation, type B neurosecretory cells in nucleus B showed signs of activation. Process of activation extended to the similar type of cells in nucleus Z after 24-48 hours. The secretion cycle in each activated cell lasted for 2 to 3 days. New Cells show sign of activation when the first group has reached the final stages in the secretory cycle. The whole trend of events in the neuro-secretory cells could be produced either by amputation of posterior segments containing ventral nerve chain or by section of the nerve chain itself. It proves that the triggering of the activity in neurosecretory cells is by the *stress stimulus carried by the injured nerves*.

#### ANNELIDA : OLIGOCHAETA

Stress and its effects on the neurosecretory system of the earthworm *Pheretima posthuma* with experimental surgical studies have been described



by Roy (1969/70). The references regarding anatomy and physiology of the neurosecretory system of *Oligochaeta* have been mentioned there.

Immediate response was noted in the a-cells after posterior amputation. Subsequently, response was noted in the b-cells. The last segmental ganglion also showed neurosecretory activity after amputation. The brain of *Pheretima posthuma* contained hormone for posterior regeneration. The brain was activated by trauma and peak activity was noted on the third day after amputation. Salt stress depleted neurosecretory substance.

#### HIRUDINEA

Neurosecretory cells have been described in the brain of the leech by B. Scharrer (1937), Hagadorn (1958), and Nambudiri and Vijayakrishnan (1958). Hagadorn (1958) found thirtysix groups of nerve cells in the brain. Twelve belonged to the cerebral ganglion and suboesophageal ganglion contained twentyfour cell groups. The ventral nerve chain contained number of segmental ganglia. Hagadorn counted total number of neurones, 2.5% of which were represented by  $\alpha$  cells and  $\beta$  cells comprised 1.5%. Neurosecretory cells were found in all the divisions of the cerebral and suboesophageal ganglia and the ganglia in the ventral nerve chain; but they are more in cell groups 4, 5, 6, 7 and 8. Legendre (1959) noted neurosecretory cells in the suboesophageal and segmental ganglia of *Hirudo* and discussed their relationships to the chromaffin cells.

From the observations of Hagadorn (1962), a relationship between  $\alpha$ -neurosecretory cells and reproductive physiology has been thought to exist.

#### SIPUNCULIDAE

Metchnikoff (1900) noted granules filling vacuoles in the cytoplasm of some giant neurones in the cerebral ganglion of *Sipunculus nudus*. Gabe (1953) observed neurosecretory cells in *Phascolion strombi*, *Golfingia vulgare*, and *Sipunculus nudus*. Akesson (1958) extended the observations on neurosecretory cells in different species of sipunculidae (Fig. 5). In sipunculus the neurosecretory substance is collected in the digitiform processes which act as a neurohaemal organ. Carlisle (1959) noted the neurosecretory axons in *Sipunculus nudus* to form loops in the digitiform processes. There is a relationship between the cycle of activity in the neurosecretory cells and the reproductive period.

#### ONYCHOPHORA

Neurosecretory cells have been studied by Gabe (1954) in five species. These cells were noted in the cerebral ganglia and ventral nerve chain. Sanchez (1958) noted neurosecretory cells in *Peripatopsis moseleyi* only. Gabe (1954) found the neurosecretory cells to form five groups in the cerebral ganglion. One single group is situated in the midline of the dorsal cellular cortex of the ganglion on the postero-dorsal part of the central body. Two laterodorsal groups are very near the intraganglionic parts of the antennal



tracts. Near the posterior segment of the cerebral trabeculae, two latero-ventral groups are located. Sanchez believed that the infracerebral vesicles act as neurohaemal organs though no direct connexion between cells and vesicles was seen. Gabe, however, could not find any direct connexion between the neurosecretory cells and the infracerebral vesicles. Gabe rather thought of the intermediate ganglion which is a collection of cells and is situated laterodorsal to the infracerebral vesicles. These cells are not neurones. There is accumulation of granules at the junction between the connective tissue sheath and the epineurium. The granules stain with chromohaematoxylin and paraldehyde-fuchsin.

## MOLLUSCA

### 1. *Scaphopoda*

Gabe (1949) observed neurosecretory cells in the anterior buccal, cerebral and pleural ganglia of *Dentalium entale*. The neurosecretory material is strongly acidophilic. Neurohaemal organ could not be demonstrated.

### 2. *Gastropoda : Prosobranchiata*

Gabe (1953) demonstrated neurosecretory cells in cerebral, pleural, and suprainstestinal ganglia in twentyfive species. No neurosecretory cell was noted in pedal and buccal ganglia. The neurosecretory cells are of small or medium size (greatest diameter  $10-20\mu$ ). The staining reaction of the secretory product with Gomori's CAHP stain or paraldehyde fuchsin varies in different animals. In all Diotocardia and in some Monotocardia, the product is acidophilic even after oxidation with permanganate and stains with phloxine.

*Personal observations in Pila globosa (Gastropoda Prosobranchia Monotocardia Taenioglossa).*

This apple-snail is the largest among the fresh water molluscs. They live in water but may invade the land.

The ganglionic masses and the connections have been depicted in Fig. 6. There are neurosecretory cells in cerebral, pleural part of pleuro-pedal, suprainstestinal and visceral ganglia. Most of the cells are  $10-20\mu$  in greatest diameter and some cells are even bigger. They contain neurosecretory material which is acidophilic in some cells and basophilic in others. Beaded appearance has been noted in the axons of some cells. These axons could be traced towards the neuropile. No neurosecretory material could be observed in the commissures and connectives.

Stress in the form of electrostimulus and saltstress was applied. Narcotization and exposure of the brain (7 animals) were done after the method of Joosse and Lever (1959). Rectangular pulses of 1 msec. duration and pulse-frequencies of 10 to 60/sec. for one to two minutes and of 5 v were used. There was depletion of neurosecretory material from the cerebral, pleural part, suprainstestinal and visceral ganglia and vacuolar formations.

### *Salt stress :*

*P. globosa* is a fresh water snail having neurosecretory cells in ganglia mentioned above. Snails (5 in each group) were placed in 0.2, 0.5 and 0.7%



NaCl-solutions for twentyfour hours. Higher salt concentrations were lethal. There was depletion of neurosecretory material from neurosecretory cells and axons in the cerebral, pleural part and visceral ganglia with vacuolar formations. Maximum change was noted in the pleural part of the pleuropedal ganglion. Boddingius (1960) noted changes in the neurosecretory cells of the cerebral ganglia of the prosobranch snail *Patella vulgata* after changes in the salt content of the medium. Lever and Joosse (1961) observed changes in the "Canopy cell" and both "droplet cells", in each lateral lobe of the cerebral ganglia of *Lymnaea stagnalis* and they lost neurosecretory material in higher salt-concentrations. In *P. globosa* there is a water balance factor which is controlled by the neurosecretory centres.

As maximum change was observed in the neurosecretory cells of the pleural part of the pleuropedal ganglia, it was thought necessary to study them by extirpation method, reimplantation method and by injection of extracts. The methods were as described by Lever *et al.* (1961) for *Lymnaea stagnalis*. *P. globosa* had increased body weight after removal of the pleural part of the pleuropedal ganglia on both sides. Lesser effects were observed after unilateral operation or after reimplantation of the ganglion after removal. Removal of the pedal part of the pleuropedal ganglion had no effect. An extract prepared from the pleural part and when injected into the body cavity through the foot had an effect on the snails regarding body weight. It was found to be diminished. This proves that the pleural part of the pleuropedal ganglion contains the water balance factor. Lever *et al.* (1961) made similar observations in the freshwater pulmonate *Lymnaea stagnalis*.

The onset of reproductive period coincides with the onset of rains in June, July and August. In the mating season neurosecretory cells in the cerebral and visceral ganglia were full of neurosecretory material and even in the form of colloids.

### 3. *Gastropoda : Pulmonata*

Description of neurosecretory cells and their end stations have been described for *Achatina fulica* and references of other workers have been mentioned there by Roy and Mishra (1972).

### • 4. *Gastropoda : Opisthobranchiata*

Berta Scharrer (1935, 1937) first discovered neurosecretory cells in *Gastropoda Opisthobranchiata*. The presence of neurosecretory cells in thirty five species of *Opisthobranchiata* was reported by Gabe (1953). The pattern of distribution was mainly in the cerebral, cerebro-pleural, pleural and abdominal ganglia. The neurosecretory cells are larger in *Opisthobranchiata* than in *Prosobranchiata*. There is pleomorphism of the nuclei. Lemche (1955, 1956) noted the distribution pattern of neurosecretory cells in nerve centres of *Cylichna cylindracea*. Giant cells and some smaller cells were thought to be of neurosecretory nature. He found neurosecretory cells in cerebral, pedal, parietal, supraintestinal, subintestinal, genital and abdominal ganglia. Such cells were not present in the pleural and the osphradial ganglia. Axonal migration of Gomori-positive neurosecretory



material was also noted and neurosecretory pathways were described by him. Bern and Hagadorn (1965) put a note of caution to such observations as lipoprotein nerve pigments are very prominent in gastropods. Similar note of caution has been put by Gabe (1966).

### 5. *Lamellibranchiata*

Gabe (1955) noted neurosecretory cells in the nerve centres of twenty species of lamellibranchs. Lubet (1955, 1956) found a definite relationship between the activity of the neurosecretory cells and that of the gonads in *Mytilus edulis* and *Chlamys varia*. With sexual maturity there was accumulation of acidophilic neurosecretory product. Depletion of neurosecretory material in the cells of cerebral and visceral ganglia was followed by the evacuation of the gonocytes. Extirpation of cerebral ganglia accelerated the discharge of gonocytes. Ablation of visceral ganglia retarded oviposition. Gabe and Rancurel (1958) noted neurosecretory cells in three species of *Teredo*. Fahrman (1961) described two types (Types I and II) of neurosecretory granules in *Unio tumidus*. Nagabhushanam (1963, 1968) recognised two types of neurosecretory cells on the dorsal surface of cerebral and visceral ganglia of *Crassostrea virginica*. One type is pyriform in shape and the secretory granules stain blueblack with chromohaematoxylin. Secretory material is also seen in the axons. The cell-type II is oval in shape and the granules and colloidal droplets are not stained by chromohaematoxylin. Rather they are stained red by Mallory's stain. This type of cell is characterized by the presence of vacuoles in the cytoplasm and even the vacuoles contain granules. Correlation between reproductive cycle and cycle of activity in type I cell could be observed. There was depletion of neurosecretory material in the oyster after electrostimulation (Nagabhushanam, 1964). Experiments were carried out by removal of cerebral and visceral ganglia. Extirpation of cerebral ganglia hastened the spawning reaction in female oysters. Mortality was highest after removal of visceral ganglia and there was increase in body weight. In visceralectomised animals injection of visceral homogenate led to rapid decrease in weight. In such animals the rates of water filtration and heart beat were considerably reduced. Antheunis (1963) described cycle of activity in the neurosecretory cells of a freshwater mussel, *Dreissena* and it was also suggested that the neurosecretory material may pass through glia cells without passing through the axons.

#### *Personal observations in a freshwater mussel, Lamellidens marginalis.*

Neurosecretory cells have been observed in the cerebral and visceral ganglia on the dorsal aspect (Fig. 6A).

The mussels were examined throughout the year. Total fixation of the soft parts was done in 1 : 10 formalin solution, Helly's or Bouin's fluid. Sections were stained with Gomori's CAHP stain, Mallory's Trichrome stain and Kluver Barrera stain. Location of cerebral ganglia was difficult to make out and so after removal of right shell valve, cerebral ganglia including portion of tissues surrounding them were removed in ablation studies. In stimulation experiments (as in *Pila globosa*) approach was made to the



labial palps including cerebral ganglia. Ablation or Stimulation studies of visceral ganglia was not difficult. The parameters of stimulation was same as in *P. globosa*. For salt-stress the mussels were put in 0.2 and 0.3% NaCl solutions for twelve hours. The neurosecretory cells were of two types (Fig. 7), their greatest diameter being 10-20 $\mu$ . Tinctorially they could be differentiated. The neurosecretory granules or substances looked blueblack with CAHP stain and these cells belong to type I as described by Nagabhushanam (1963, 1968). The neurosecretory material in type II cells did not look blueblack with CAHP stain. It appeared red with Mallory's stain. Vacuolisation in the cytoplasm of both types of cells could be found. Luxol fast blue-positive materials were found in both types but more in type I. Axonal migration could be observed for a very short distance towards the neuropile. No true neurohaemal organ could be observed. Secretory cycle could be established. There were some cells with marginally placed scanty granules. In others the granules were plenty but one is separated from the other. In the third type there was confluence of the granules to form colloid-like picture. In the fourth type there were vacuolar formations in the cytoplasm indicating discharge of neurosecretory material.

In the reproductive period (June, July and August) the neurosecretory cells in the cerebral and visceral ganglia were full of neurosecretory material.

After removal of the ganglia, the mussels lived only for two days. So, all experimental observations had to be curtailed upto two days (though a very short period of observation). Hastening of spawning reaction (Fig. 8) was noted after removal of cerebral ganglia in females.

After removal of the visceral ganglia, there was increase in body weight and after injection of the extract of visceral ganglion, the body weight diminished. Reimplantation of the visceral ganglia had no detrimental effects. This indicates that the visceral ganglia may contain a type of water balance factor. This is also proved by the salt-stress studies. Depletion of neurosecretory material (Fig. 9) has been noted more in the cells of the visceral ganglia than those of the cerebral ganglia. Vacuolar formations were maximum. Similar feature was noted in stimulation experiments.

#### MOLLUSCA : CEPHALOPODA

The epistellar bodies of octopods and the parolfactory vesicles of decapods may have neurosecretory cells and endocrine function. Electron microscopic studies of the epistellar body could not detect any neurosecretory activity in the subjacent neurones and it appeared to be a rudimentary photoreceptor by Nishioka *et al.* (1962).

#### MATURATION OF THE GONADS IN THE OCTOPUS —A NEUROENDOCRINE CONTROL

Control of reproduction by nerve centres could be observed by Boycott and Young (1956) in female octopuses. After removal of the vertical lobes of the brain, sexual maturity was noted in some young female octopuses and they laid eggs. With the removal of the vertical lobe, a small centre



beneath it is also removed. From this centre a nerve proceeds to the optic gland. The glands enlarge and are of deep orange in colour. The cells of the glands enlarge and are filled up with secretion.

Bonichon (1967) studied neurosecretory phenomenon in *Octopus vulgaris*. Characteristic changes by an increase of the cell-size, nuclei and cytoplasm of the optic glands during sexual maturation were observed in the optic glands. Secretory products were noted in the cytoplasm and the cytoplasmic processes of the cells. The embryos and the larvae do not possess the optic glands. The glands are noted in very young octopus. The glands have a nervous origin and possibly they develop from cells of the olfactory lobe. Cytoplasmic fuchsinophilic inclusions (about  $1\mu$ ) have been noted in the olfactory lobe neurones during growth and sexual maturation. The number of inclusions decrease in a female during laying. Bonichon (1967) thought of a possible relationship in the function between the olfactory lobe and the optic gland. Neurosecretory neurones could be found in the ventral and posterolateral parts of the visceral lobe. They were homologized with the cells of the ventral layer of the nervous system of the vena cava. Neurosecretory activity could be observed in very young male and female Octopus. Bonichon (1967) could observe penetration of a nerve into the optic gland.

The optic glands are found in all cephalopods except *Nautilus*. The optic glands are situated on the optic stalks (Fig. 10). These are pale yellow bodies. The glands can very easily be confused with olfactory and peduncle ganglia (Fig. 11). In octopus there are changes in the optic glands with enlargement of the ovary after section of optic nerves or lesions of the subpedunculate lobe in the brain. After such lesions the glands become light orange in colour and their size increases by more than ten times.

The arrangement of the cells in the optic glands is in solid masses. Vesicular formation is not noted. There are two types of cells—(1) chief cells and (2) small supporting cells. The nerve supplying the optic glands takes its origin in the subpedunculate lobe. Wells and Wells (1959) state that in enlarged optic glands the supporting cells do not change and they conclude that these cells do not take part in secretion. The chief cells increase in volume with enlargement of the glands. There is enlargement of the nuclei, nucleoli and nuclear granules. The cytoplasm increases in volume and is vacuolated.

Wells and Wells (1959) found that the secretion of the optic glands in Octopus controls the maturation of the gonad. These glands have an inhibitory nerve supply from the subpedunculate lobe of the brain. Experiments leading to enlargement of optic glands with maturation of the gonad are diagrammatically reproduced in figures 12 to 14 and 16 after Wells and Wells.

Wells and Wells (1969) thought that in the male Octopus the optic glands are controlled through the subpedunculate lobe by the testis (or testicular ducts). There is prolonged maturity of males compared with females. The negative feedback system would explain the sexwise difference. In females there is apparently no ovarian hormone. When the inhibitory influence of the subpedunculate lobe is removed in females, the optic glands



are released to produce the gonad-maturing-hormone in an all—or—nothing manner.

The male cephalopod gonad-subpedunculate lobe-inhibitory nerve-optic gland-axis can be compared to hypothalamus-anterior pituitary-gonad-hypothalamus of the mammals. In the cephalopod the link is inhibitory neural, whereas it is neurovascular (hypophyseoportal vessels) in the mammals. The optic gland system in the cephalopod is bilateral.

Nishioka *et al.* (1970) tried to elucidate the nature of the nerve to the optic gland by light, fluorescence and electron microscopy. The optic gland of *Octopus* and *Loligo* is composed of stellate cells with axon-like processes and glial elements. Aminergic (?) nerve with considerable fluorescence emerges from the brain and enters into the optic gland in association with capillaries. Small, adrenergic-type, dense-cored granules were observed in most nerve fibre profiles. Larger electron-dense granules were found in other nerve fibres. No synaptoid structures were visible, although nerve fibres abutted against stellate cells. Thus the control of the gland by the nerve was thought to be due to diffusion of the inhibitory substance from the nerve fibres and acting on the secretory stellate cells.

#### ECHINODERMATA

Some kind of neurosecretory activity could be found by Unger (1960) in radial nerves and circumoral nerve ring of some Asteroids (Fig. 17). Neurosecretory cells have been described in ophiurids and asteroids by Fontaine (1962).

#### ARTHROPODA XIPHOSURA

B. Scharrer (1941) described neurosecretory cells in the circumoesophageal nerve ring and the ventral nerve cord of *Limulus*. There was increasing number of such cells in the posterior part of the nerve ring than the anterior one. Increasing number of cells was noted in larger animals than in younger individuals. No correlation could be made between the number of cells and the annual cycle or any sexual difference. Neither there was any relationship with circadian cycle. The very big cells have the nucleus pushed to the periphery by large secretory colloid and there are vacuoles situated peripherally. Axonal migration of the secretory product was not noted.

Brown and Cunningham (1941) studied the distribution of chromatophorotropins in the nervous system of *Limulus*. The extracts were tested on eyestalkless *Uca* where there was darkening reaction. Maximum chromatophorotropic activity could be located in the posterior part of the perioesophageal ring where there was maximum number of neurosecretory cells.

Waterman and Enami (1954) studied neurosecretion in the lateral rudimentary eye of *Limulus* and *Tachypleus tridentatus*. Secretory cycle has been noted in the sensory cells of the lateral rudimentary eye. The neurosecretory material is discharged into the connective tissue surrounding



the cells. From this place the material is transported by humoral path. Extracts of these organs from *Tachypleus* had chromatophorotropic effects on young *Sesarma haematocheir* but extracts similarly prepared from *Limulus* were without effect on the chromatophorotropins of *Uca*. According to Waterman and Enami (1954) the lateral rudimentary eye of xiphosura could be compared to the X-organ-sinus gland system of decapod crustaceans or pars intercerebralis- corpora cardiaca system of insects.

## ARACHNIDA

### A. SCORPIONIDA

Neurosecretory phenomena have been studied by Gabe (1955). Two symmetrically placed groups of neurosecretory cells are located in the dorsal and aboral part of the protocerebrum. Neurosecretory cells have also been detected in the suboesophageal ganglion but no neurohaemal organ has been described. The axonal paths of the protocerebral neurosecretory cells are along the (a) intestinal and (b) lateral nerves of Police (1903). The neurohaemal organ is represented by the "*Stomatogastric ganglia*" of Police. This organ consists of (a) neurosecretory endings stainable with chromehaematoxylin in the Gomori method and a deep violet with paraldehyde fuchsin ; (b) Ovoid polyhedral cells containing strongly acidophilic secretory product. This acidophilia is not changed to basophilia even after oxidation with permanganate.

### B. PSEUDOSCORPIONIDA

Protocerebral neurosecretory cells and cells in suboesophageal ganglion have been described by Gabe (1955). Axon paths from the protocerebral neurosecretory cells proceed in an oral direction and end in *parapharyngeal organs*. The structure of this organ is same as is noted in the organ of Police.

### C. OPILIONES

Neurosecretory process has been described by Gabe (1954, 1955), Herlant-Meewis and Naisse (1957) and Naisse (1959, 1961). The protocerebral neurosecretory cells are situated in two oral and two aboral groups. The axonal paths from the oral groups form a curve with concavity directed dorsally and aborally and after joining the stomodeal bridge proceed backwards along the oesophagus. The pathways from the aboral cell groups are more or less similar but the curvature has a smaller radius and they proceed aborally on the two sides of the median trachea. At the aboral border of the cerebral ganglion the two nerves on each side join together and form a neurohaemal organ on each side to be named as *Gabe's paraganaglionic plaques*. The structure of this plaque is same as is noted in the neurohaemal organ of *scorpionida*. Neurosecretory cells have been described in the suboesophageal nerve mass.

Products formed in the cells of the oral group are necessary for the course of the moult cycle. For the maturation of the gonocytes, products in the cells of the aboral group are responsible.



### D. ARANEIDA

Neurosecretory systems in different genera of spiders have been studied by Gabe (1955), Legendre (1959) and Kuhne (1959). Neurosecretory cells showing secretory cycle have been noted in a pair of oral and another pair of aboral groups in the brain (Fig. 18). Other cerebral cell groups have also been reported. Neurosecretory cells are also found in (a) ganglia of pedipalps (b) ganglia of walking legs and (c) ganglia of abdominal nerve mass. *First and second organ of Schneider* forms the neurohaemal organs.

The first organ of Schneider consists of (a) bipolar neurones, (b) glandular cells with acidophilic products and vacuoles and (c) neurosecretory endings bearing secretion products from neurosecretory cells of the brain. The second organ of Schneider is smaller than the first one and contains only gland cells with acidophilic secretion. The marginal ganglion of Schneider consists of (a) Schwann cells and (b) secretory products which are same as noted in first organ of Schneider.

Process of neurosecretion has been related to the maturation of the gonocytes, age and course of hibernation.

### E. ACARINA

Gabe (1955) described four groups of neurosecretory cells, two on each side of the protocerebrum. The axonal paths end in the "*paraganglionic plaques*" which join in the midline and act as a neurohaemal organ. These organs contain acidophilic polyhedral cells and neurosecretory products accumulate in the organs. Neurosecretory cells were also noted in the suboesophageal nerve mass. Axonal migration of neurosecretory material and end organs could not be detected in relation to these neurosecretory cells.

## ARTHROPODA MYRIAPODA DIPLOPODA

Protocephalic neurosecretory cells, paths and endorgans have been described by Gabe (1954), Palm (1956), Sahli (1958, 1961) and Prabhu (1959, 1961, 1962) (Fig. 19). The neurosecretory cells are near the globuli I and II. The neurosecretory product is stainable with chrome haematoxylin after Gomori's method or with paraldehyde-fuchsin. There are tritocerebral neurosecretory cells and neurosecretory phenomena have been observed in the suboesophageal ganglion and the ventral nerve chain. The neurohaemal organs are (1) the cerebral gland, (2) the hypocerebral organs in *Julidae* and (3) the connective body in *Jonespeltis*.

### THE CEREBRAL GLAND

In *Glomeris marginata* it is of digitate appearance at the end of the nerve. The digitations contain neurosecretory endings and small rounded or polyhedral cells with acidophilic secretory products. The cerebral gland is spindle-shaped in *Polydesmus*. The *Julidae* have globular cerebral glands.



## HYPOCEREBRAL ORGANS IN JULIDAE

These are collections of cells and neurosecretory material. The neurosecretory material is of cerebral origin. The cells show evidence of secretory activity.

### THE CONNECTIVE BODY IN JONESPELTIS

It contains neurosecretory endings from tritocerebral neurosecretory cells surrounded by Schwann cells.

Seasonal variation in the activity of the neurosecretory cells was observed by Prabhu (1962).

## CHILOPODA

The Protocephalic neurosecretory pathway in four different orders *e.g.*, Geophilomorpha, Scolopendromorpha, Lithobiomorpha and Scutigermomorpha was described by Gabe (1952, 1953, 1956), Palm (1956) and Scheffel (1961). Neurosecretory cells are found also in the suboesophageal nerve mass and the ventral ganglia. The protocephalic neurosecretory cells send axons to the cerebral gland which acts as neurohaemal organ *via* cerebral gland nerves (Fig. 20). The cerebral gland has neurosecretory endings and cells elaborating acidophilic secretions. The neurosecretory material stains with chrome haematoxylin in Gomori's method and with paraldehyde-fuchsin. Thus the neurosecretory endings and the cells in the cerebral gland have different tinctorial affinities.

Palm (1956) thinks that the protocephalic neurosecretory cells are responsible for the development of adult characteristics. There are evidences to suggest that cerebral glands are involved in molting (Joly, 1962).

## SYMPHYLA

Protocerebral neurosecretory cells and cerebral glands have been described by Juberthie—Jupeau (1961) in *Scutigrella pagesi*.

## CRUSTACEA : ENTOMOSTRACA

The neurosecretory systems have been described in Branchiopoda by Lockhead and Resner (1958), Menon (1962), in Cladocera by Sterba (1957), in Copepoda by Carlisle and Pitman (1961), in Ostracoda by Weygoldt (1961), in Cirripedia by Barnes and Gonor (1958).

## CRUSTACEA : MALACOSTRACA

In connexion with the optic ganglia situated in the eyestalks there are groups of neurosecretory cells. Of the groups there are two types of "X-Organs"—the ganglionic X-organ and the sensory pore X-organ. On the medulla terminalis of brachyurans the two types of X-organs are fused together but in natantians they are separate. Neurosecretory cells are



also noted in the brain, the connective ganglia, the tritocerebral commissure and thoracic ganglia. The neurosecretory cells in the eyestalks have neurosecretory materials which can be stained by Gomori's chromealum-haematoxylin and paraldehyde-fuchsin. These cells have been noted by Carlisle and Passano (1953) in *Crangon vulgaris* and *Lysmata Seticandata*, by Carlisle and Knowles (1959) in *Leander serratus* by Carlisle (1959) in *Pandalus borealis* and by Drach and Gabe (1962) in *Athanas nitescens*. Roy (1958) noted neurosecretory cells in the brain, eyestalk and thoracic ganglion of *Palaemon carcinus*. Under dark field illumination highly refractile substance was found in the cells, axons and the sinus gland. The neurosecretory substance could be stained by Anilineblue or by Gomori's CAHP method. Majority of the neurosecretory substance took up haematoxylin component of the Gomori's colour but some took up the phloxine component. The X-organ of the *Palaemon carcinus* is composed of big monopolar cells and rounded cells are found in groups. Cells of syncytial pattern are also found. The X-organ can be divided into a pars distalis part which is a storage centre and a pars ganglionaris part which is a productive one. The two parts are joined by a nerve. The sinus gland is another storage centre. When the sinus gland is removed, there is accumulation of neurosecretory substance in the proximal stump. Light can directly control the neurosecretory centres in the brain by penetration through the more or less transparent structures above the brain. It was also found that watery extracts of eyestalks, brain and thoracic ganglion give rise to depletion of sudanophilic substance in the suprarenal gland of *Bufo melanostictus*. This is an example of invertebrate neurosecretion acting on vertebrate target organ.

Carlisle (1959) noted five cell types in the organ of Hanstrom (pars ganglionaris of the X-organ) and X-organs of the medulla externa and medulla interna.

Durand (1956) reported four types of neurosecretory cells in *Orconectes virilis*.

In the brachyuran *Sesarma*, Enami (1951) described three types of neurosecretory cells. Three different types were also described by Bliss *et al.* (1954) and Parameswaran (1956) in brachyura. Potter (1958) noted six cell types and Matsumoto (1954, 1956, 1958) observed eleven different cell types.

### THE SINUS GLAND

The sinus gland is a neurohaemal organ. Hanstrom (1937) found different types (anatomical) of the sinus gland. This may be of inversion type or cup shaped or eversed type. Neurosecretory fibres end in the gland from (1) ganglionic X-organ of medulla terminalis, (2) neurosecretory cells of other optic ganglia, and (3) neurosecretory cells of the brain, the connective and thoracic ganglia. The sinus gland consists of neurosecretory endings, neurosecretory material and cells. Bliss and Welsh (1952) concluded that sinus gland stores and discharges neurohormones which are derived from the different neurosecretory cells. Gabe (1954) thinks that the sinus gland cells have secretory activity and act as gland cells producing



secretion. Hodge and Chapman (1958) found similarity of these cells with the pituicytes of the neurohypophysis of the vertebrates. They also considered the influence that these cells exert on the secretory processes of the neurosecretory axon endings.

Different types of secretory products have been noted in the sinus gland by Potter (1954, 1958) with specific distributory patterns. Six such zones have been noted in the ganglionic X-organ of *Callinectes sapidus*. Rehm (1959) noted six types of nerve terminations in the sinus gland of *Carcinus maenas*.

#### *The pars distalis X-organ or the sensory-pore X-organ.*

It consists of (1) bipolar sensory cells, (2) round or elongated epithelioid cells and (3) lamellated "Onion bodies." The onion bodies are secretion products but there is a controversy regarding their origin. One view supports their production in the sensory pore X-organ. Carlisle (1953, 1959), Knowles and Carlisle (1956) and Carlisle and Knowles (1959) think in a different way. According to them, the onion bodies are in reality the axonal endings of neurosecretory cells of the brain and of the ganglionic X-organ of the medulla terminalis. Collectively the axons form the connexio X-organii.

Gabe (1966) interpretes the onion bodies as of intracellular nature and a secretory cycle has been well documented. This is contradictory to the idea of "Swollen nerve endings."

#### POSTCOMMISSURE ORGANS

In the thorax of crustaceans, chromactivating hormones have been found to be contained in the central nervous tissues by Brown (1935) and Knowles (1939). The greatest concentration of the hormones were found to be located in the circumoesophageal connectives and postoesophageal commissure (Brown, 1946 and Knowles, 1951, 1953). Knowles considered these organs as neurohaemal areas. Carlisle and Knowles (1959) described the postcommissure organs in *Penaeus*, *Palaemon* and *Squilla*. The nerve cell bodies of this organ is thought to be located in the tritocerebrum. Chromatophorotropins have been obtained from the postcommissure organs.

#### PERICARDIAL ORGANS

Alexandrowicz (1952, 1953a, b) described the pericardial organs. The organs have been found in the decapod and stomatopod crustacea. These consist of ramifications of nerve endings. In the *Squilla* they are located in the dorsal blood sinus and in the decapoda the location is in the pericardium. Anatomically they are either plexiform or lamellated. Maynard (1961a, b) studied the pericardial organs in *Brachyura*. Neurosecretory cells were found in the thoracic ganglionic mass and in the pericardial organs. Axons from the ganglionic mass terminated in the organs and some axons were directed towards the anterior pericardial ramifica-



tions via the segmental nerves. In *Brachyura* the organs act as thoracic neurohaemal organ.

The pericardial organs contain neurohormones controlling the heart-rate and amplitude of the heartbeat.

Knowles (1962) studied the ultrastructure of the pericardial organs of *Squilla mantis*. Two types (A and B) of neurosecretory fibres were noted. There were three types of cells: (1) Schwann cells, (2) glia-like cells, and (3) secretory cells.

The endocrine glands in the crustacea are the Y—organs (Gabe, 1953, 1956), androgenic glands and ovary.

The neurosecretory systems are responsible for (1) colour change, (2) retinal pigment migration, (3) molting, growth and regeneration, (4) reproduction, (5) metabolism and (6) control of heart rate.

#### REGENERATION OF THE AMPUTATED LIMB IN MALACOSTRACOUS CRUSTACEA : A NEUROENDOCRINE CONTROL

Bliss (1960) discussed this problem. Regeneration of the missing limb takes place in three different stages and Bliss (1956) named them as basal growth, plateau and pre-ecdysial growth. Bliss (1962) discussed the neuroendocrine system controlling the growth and moulting in the land crab *Geocarinus lateralis*. Unfavourable factors (extreme temperature, inadequate moisture, community life and light) lead to the release of moult-inhibiting hormone from the sinus glands. This leads to inhibition of Y organs and there is no release of Y organ hormone (moult-promoting hormone). Thus there is delay in growth of limb buds and delay in moulting. Favourable factors (moderate temperature, adequate moisture, solitary life and darkness) or eyestalk removal give rise to the release of moult promoting hormone and there is rapid growth of limb buds and moulting without delay.

Durand (1960) studied the regeneration process of the lost limbs in the crayfish. Type 2 cells of the ganglionic X-organ were involved in the process. During the basal growth period after amputation, these cells contained plenty of small granules stained by paraldehyde-fuchsin. At the plateau stage there were few large secretory granules in these cells. There was disappearance of these granules in the pre-ecdysial growth period. Durand noted changes also in the Y—organs.

On stimulation (electric) of the ganglionic X-organ in *Palaemon carcinus* there is depletion of neurosecretory material (stainable with Gomori's chrome alum haematoxylin) with formation of vacuoles. There is depletion also of the same material from the sinus gland.

Stress incites the process of regeneration of a missing limb or changes in the neurosecretory cells after electrostimulus. In the former process the events are slow to develop whereas in the latter one, successive trends of events are quick. In both situations there is an empty cell at the end, indicating liberation of the neurohormone. Hyperglycaemia occurs after stress. Same feature happens after injection of extracts of eye stalks or X-organ-sinus gland extracts in normal animals.



## INSECTA : APTERYGOTA

## DIPLURA

There is no frontal organ in *Japyx*. The neurosecretory cells are located in the pars intercerebralis. The neurosecretory material can be stained by Gomori's CAH and with paraldehyde-fuchsin. The axonal paths (nervus corporis cardiaci I) cross and after leaving the cerebral ganglion enter the corpora cardiaca. The allatal nerve enters the corpora allata.

## THYSANURA

The neurosecretory cells are situated in a pair of frontal organs which are separate from the cerebral ganglia. The neurosecretory material can be stained by Gomori's CAH and paraldehyde-fuchsin. The axonal paths (nervus corporis cardiaci I) cross and enter the corpora cardiaca after they come out of the cerebral ganglion.

The corpora cardiaca consist of the neurosecretory endings and cells. The secretory product of the cells can be stained by phloxine component of Gomori's CAHP stain.

## INSECTA : PTERYGOTA

Neurosecretory system in insects has been dealt with by Gabe (1953, 1954), Scharrer and Scharrer (1954), B. Scharrer (1955), van der Kloot (1960), Knowles (1963), Bern and Hagadorn (1965), Gabe (1966) and others.

The neurosecretory cells are located in the pars intercerebralis in paired groups (Fig. 21). The neurosecretory material is carried along the axonal paths and collect in the corpus cardiacum from where it is discharged into the body fluid. Some neurosecretory axons pass through the corpus cardiacum to end amidst the cells of the corpus allatum. The neurosecretory material is stainable by Gomori's chrome haematoxylin and paraldehyde-fuchsin.

The axonal paths from the neurosecretory cells in the brain are known as nervi corporis cardiaci I, which cross with the fellow of the opposite side in the midline and after emerging from the brain adopt an extraganglionic course to enter the corpus cardiacum.

The second group of neurosecretory cells is located near the globuli-cells of the corpora pedunculata. The axonal paths from this cell group form the nervus corporis cardiaci 2, which run a straight course backwards without any crossing and then enter into the corpus cardiacum (Fig. 22).

De Lerma (1956) thinks that when the neurosecretory granules are formed, they are phloxinophilic. Subsequently with the maturation, the granules can be stained with Gomori's chrome haematoxylin. Highnam (1961) observed in *Schistocerca gregaria* that the neurosecretory granules could be stained sometimes with Gomori's chrome haematoxylin or paraldehyde-fuchsin and at other time with phloxine.



The corpus cardiacum contains the neurosecretory endings of the protocerebral neurosecretory cells and thus it acts as a neurohaemal organ. It also contains glia cells, nerve cells and intrinsic glandular cells.

Neurosecretory cell stations have also been noted in the tritocerebrum with corpus cardiacum as the end station. Neurosecretory cells are also present in the suboesophageal ganglion and thoracoabdominal nerve chain.

B. Scharrer (1962) described the ultrastructure of the neurosecretory system of the insect *Leucophaea maderae*. In various zones of the neurosecretory neurones, electron dense granules and vesicles of 1000 to 3500 Å in diameter have been observed.

Neurosecretion is important in the insects in postembryonic life, ecdysis, metamorphosis, diapause, metabolism, reproduction, rhythm of activity, and colour adaptation.

#### STRESS AND NEUROSECRETION IN INSECTS

Huber (1965) discussed neural integration (central nervous system) in insects. Huber (1962) noted the following after ablation of parts of the brain in the grasshopper (Figs. 23, 24). There is no interruption of sound production after removal of one calyx but the jumping activity in the male is increased at 27%. Sound production is stopped after removal of two calyces but jumping activity is increased further. Sound production is interrupted after destruction of the whole central body and there is depression in jumping activity after an increase at the beginning. The initial increase is due to the operation.

Changes in behavioral pattern were studied in unrestrained insects by Huber and others after application of electric stimulus through chronically implanted electrodes in different parts of the brain (electrodes made of tungsten, steel or platinum wires of 10-30 $\mu$ , insulated to the tip; monopolar or bipolar; rectangular pulses of 1 msec. duration with frequencies of 10-60/sec.).

Hodgson and Geldiay (1959) observed diminution of neurosecretory material in the corpora cardiaca of *Blaberus carnifer* after electrical stimulation or violent muscular exercise.

Highnam (1961) used high-frequency electrical shocks (10 v., 40 pulses/sec. for 15 minutes or longer) through the optic nerves of two week-old female desert locust (*Schistocerca gregaria*), reared without males. Forty-five minutes after the shocks there was almost complete depletion of stainable material from the neurosecretory systems. Low-frequency (10 v., 40-75 pulses/min. for 15-30 minutes) electrical shocks applied through the ventral nerve cord, the surface of the brain or through the optic nerves manifested with increased amount of the material along the nervi corporis cardiaci I and also in the storage areas of the corpora cardiaca when compared with the operated controls. Low and high frequency stimulation illustrates the stages in the movement of the neurosecretory material along the nervi corporis cardiaci I and into the corpora cardiaca. Highnam



(1961) also applied stress to the insects (two week-old females reared without males) by rotation in a flask for 45 minutes in such a way that they were continually turned upside down and the animals continually struggled to obtain their normal position. There was depletion of neurosecretory material in the neurosecretory systems.

The picture of neurosecretory material during copulation is same as observed in the low-frequency stimulation group. Twelve hours after copulation the picture resembled that following high frequency electrical stimulation and induced activity.

B. Scharrer and Kater (1969) observed discharge of neurosecretory material in *Periplaneta americana* from corpus cardiacum after electrical stimulation of the nervi corporis cardiaci. The active material is discharged from parts of the neurosecretory axons which are preterminal morphologically. These sites of release are known as *synaptoids*. These are transient structures formed according to demand. Exocytosis is the method of release in some arthropods (Normann, 1969 ; Weitzman, 1969).

Normann and Duve (1969) noted release of neurosecretory granules from the intrinsic neurosecretory cells of the corpus cardiacum by electrical stimulation of the brain provided the nerve connecting the brain with the corpus cardiacum was intact.

Normann (1970) observed the mechanism of neurohormone release in *Calliphora erythrocephala*. The stimulus current was of 5-15  $\mu$ A, 0.5 msec. duration, 5 V rectangular pulses, 3/sec., for 30-45 seconds. The blowflies were stressed also by shaking them in a flask for several minutes. Axonal depletion of neurosecretory granules was noted after the shaking stress. Depolarization with acetylcholine and esserine or with potassium was also attempted. Electron microscopic picture of small vesicles at the axolemma represents *synaptoid contacts*. These swarms of vesicles differ from the synapses and represent fragmentation products of granule membranes during exocytosis.

Changes in the neurosecretory system of *Iphita limbata* was observed by Nayar (1960) in water loading and dehydration conditions. Nayar (1962) stated further about the works of Menon in similar directions of *Periplaneta*. Twenty-four hours after injection of 1% sodium chloride solution there was disappearance of neurosecretory colloids from the corpora cardiaca. There was retention of colloids in the glands, twenty-four hours after injection of distilled water. Retention of the colloids increased further when additional distilled water is injected 24 hours later.

## CONCLUSION

From the findings above and discussions, it is evident that the stress responses occur even in invertebrates. These responses are mediated by the neuroendocrine system. The neurosecretory hormones are released from the neurosecretory cells or from the neurohaemal organs. These hormones act upon the endocrine organs where present or upon the tissue elements in order to achieve well-being of the animal in the form of tiding over a critical situation. When the critical period is over, the neuro-



secretory systems reach a basal level of activity until they are reactivated due to some other forms of demands, as for example, reproduction and others. Thus *homeostasis* which happens in vertebrates also has its counterpart in invertebrates involving the neurosecretory systems. All the endocrine organs and hormones in invertebrates have not been discovered fully. A time is approaching fast when the anatomical and physiological aspects of them will be unveiled properly and we will be able to understand them in a better way. Scharrer and Weitzman (1970) have rightly said, "The most impressive result of the development of appropriate mechanism throughout the animal kingdom is the neurosecretory neuron, a cell which is highly specialised and at the same time capable of performing multiple tasks," and studies in invertebrates with neurosecretory systems will lead to further development of neuroendocrinology.

#### INVERTEBRATE NEUROSECRETORY SYSTEMS ANALOGOUS WITH THOSE FOUND IN VERTEBRATES (Bern, 1971 and others)

##### *Invertebrate*

- (1) In oligochaeta and hirudinea the neurohaemal region is found at the posterior part of the brain (supraoesophageal ganglion).

In gastropod molluscs neurohaemal zones are on surface of ganglia, connectives and nerves.

Neurosecretory axons end in the wall of blood vessels to release neurosecretion (the vena cava system of cephalopods).

Neurosecretory axons enter the aortic wall of heteropteran insects.

- (2) Corpus cardiacum of insects and sinus gland of crustaceans are end stations.

- (3) Cephalopod optic gland (reproductive hormones) is inhibited by aminergic 'B' type of fibres from subpedunculate lobe.

##### *Vertebrate*

- (1) The hypothalamic neurosecretory cells project to the median eminence to discharge neurosecretion into the primary capillary plexus of the hypophyscoportal system.

The caudal neurosecretory system in elasmobranchs where the neurosecretory endings collect ventrally in the spinal cord with rich vascular supply.

- (2) End stations are neurohypophysis and urophysis in the teleost fish.

Neurosecretory product may be discharged into cerebrospinal fluid.

- (3) The prolactin-secreting cells of the rostral pars distalis in the teleost fish is inhibited by aminergic 'B' type of fibres from nucleus lateralis tuberculi.



*Invertebrate**Vertebrate*

- |  |  |
|--|--|
| <p>(4) The crustacean sinus gland, postcommissure organs and pericardial organs and Cerebral neurohaemal areas in oligochaeta and hirudinea and Insect perisymphatic organs.....have no associated endocrine tissue.</p> | <p>(4) Teleost urophysis has no associated endocrine tissue.</p> |
|--|--|

## ASSOCIATION OF ENDOCRINE TISSUE WITH NEUROHAEMAL ORGAN

- |   |  |
|---|--|
| <p>(5) (a) Juxtaganglionic organ in mollusca : gastropoda, prosobranchiata, opisthobranchiata.</p> <p>(b) Mediodorsal bodies and similar bodies (cell groups) in the sheath of visceral ganglion of gastropoda : pulmonata.</p> <p>(c) Optic glands in mollusca : cephalopoda.</p> <p>(d) Infracerebral gland in annelida : polychaeta.</p> <p>(e) Intrinsic cells of cerebral gland in arthropoda : myriapoda.</p> <p>(f) Intrinsic cells of Schneider's organ in arthropoda : arachnida.</p> <p>(g) Corpus allatum and intrinsic cells of corpus cardiacum in arthropoda : insecta.</p> | <p>(5) Neurohypophysis is associated with adenohypophysis.</p> |
|---|--|

## ELECTROPHYSIOLOGY

*Invertebrate**Vertebrate*

- |   |  |
|---|--|
| <p>(6) Neurosecretory neurones in molluscs, annelids, insects and crustaceans can conduct impulses.</p> | <p>(6) Neurosecretory neurones can conduct impulses in hypothalamus and caudal neurosecretory systems.</p> |
|---|--|



*Invertebrate**Vertebrate*

- |  |   |
|--|---|
| (7) Action potentials are of long durations.   | (7) Action potentials are of long durations. In mammals identical action potentials have been recorded from neurosecretory and non-neurosecretory neurones. |
| (8) Neurosecretory neurones are innervated by cholinergic and aminergic neurones.                                | (8) Neurosecretory neurones are innervated by cholinergic and aminergic neurones.   |
| (9) Mechanism of release from neurosecretory neurones after stimulation is similar to that noted in vertebrates. | (9) Mechanism of release from neurosecretory neurone after stimulation is similar to that noted in invertebrates.   |

## STRESS

- |   |  |
|---|--|
| (10) Electrical stimulus leads to depletion of neurosecretory material in molluscs, insects and crustacea from cell and end stations. | (10) Electrical stimulus leads to release of neurohormones from neurohypophysis and urophysis. |
| (11) Neuroendocrine systems respond to stress and maintain <i>homeostasis</i> .   | (11) Neuroendocrine system respond to stress and maintain <i>homeostasis</i> .                 |

## II. RECENT PROGRESS IN THE STUDY OF CAUDAL NEURO-SECRETORY SYSTEM OF FISHES

The caudal neurosecretory system is found in the posterior part of the spinal cord of elasmobranch and teleost fishes. It has got cell station, axonal paths and neurohaemal areas or organs. The organs are known as urophysis in the teleost fishes.

The caudal neurosecretory system has been studied and reviewed by many Enami, 1959 ; Sano, 1961 ; 1964 ; Roy, 1962 ; Bern and Takasugi, 1962 ; Holmgren, 1964 ; Bern *et al.*, 1965 ; Arvy, 1966 ; Gabe, 1966 ; Fridberg and Bern, 1968 ; Bern, 1969 ; Chester Jones *et al.*, 1969 ; Chan *et al.*, 1969 ; Lederis, 1970 ; Lederis *et al.*, 1971 ; Chan, 1971 and others.

Roy (1962) noted that zinc may act on the caudal neurosecretory system with discharge of neurosecretion which activates the pituitary-adrenal-axis of the fish. In the figure number 22 on page 23 it was shown that nervous and humoral stimuli activate caudal neurosecretory cells, which could lead to the discharge of osmoregulatory hormone/s. Neuro-



secretion from neurohypophysis caudalis could act on corpuscle of Stan-  
nius with discharge of osmoregulatory hormone/s or hormones acting on  
reproductive system. Neurosecretory hormone from the neurohypophysis  
caudalis could act also on adrenocortical cells.

Histological and experimental observations on the caudal neuro-  
secretory system of some Indian fishes were made by Roy (1962). From  
the caudal spinal cord including the terminal swelling (caudal neurosecre-  
tory system) of the Indian teleost fish *L. rohita*, a material (Peptide) has  
been isolated which stimulates the release of ACTH *in vivo*. This does  
not contain histamine or any vasopressor, antidiuretic or oxytocic activity.

Roy (1964) noted increased plasma 17-OHCS levels after the injec-  
tions of diencephalic and caudal neurosecretory extracts in nonhypo-  
physectomized *O. punctatus*. The extracts were taken either from *O. punc-  
tatus* or from *L. rohita*. There was no increase in the response when the  
extracts were injected into hypophysectomized *O. punctatus*. This proves  
that pituitary is essential for the mediation of the response. Histologically  
there was accumulation of nsm in the storage-release-center, *i.e.*, the uro-  
physis, after hypophysectomy in *O. punctatus*. This feature was not noted  
after autogenous hypophysial grafts in the anterior chamber of the eye.

The neurosecretory material in the caudal neurosecretory system  
has strong affinity for acid dyes, *e.g.*, phloxine, azan and fuchsin. Acid-  
violet method of Takasugi and Bern (1962) is a very good stain for this  
purpose. Alcian blue method does not suggest the presence of disulphide  
groups in the urophysis. The Gomori-negative neurosecretion of the caudal  
neurosecretory system is composed mainly of the elementary neurosecretory  
granules in the size-range of 1000 to 3000 Å (Bern and Takasugi, 1962).  
Golgi apparatus in the perikaryon appeared to organize these granules.  
Sano and Knoop (1959) made the first electron-microscopic studies of  
neurosecretory cells. Association of the elementary granules with the  
Golgi apparatus was noted in the Dahlgren cells of *Tinca vulgaris*.

Fridberg *et al.* (1966) studied the caudal neurosecretory system of  
the isospondylous teleost, *Albula vulpes* from open sea and oceanic ponds  
with wide variations in salinity. This animal has got a well-developed  
lobate urophysis and extraurophysial neurohaemal regions as is seen in  
elasmobranchs. In the pond fish the neurosecretory cells and the neuro-  
haemal areas and organs have greater amount of neurosecretory materials.  
Urophysial system has got an osmo (iono)-regulatory role. Some neuro-  
secretory neurones have processes which enter into the central canal of  
the spinal cord and also send a process into the urophysis. There are  
two types of neurosecretory cells (caudal) as can be differentiated by stain-  
ing reactions and by the size of the elementary neurosecretory granules.  
Elementary neurosecretory granules are formed by the Golgi complex  
and also by transformation of Golgi vesicles into granules. Local form-  
ation of vesicles and granules in the neurosecretory terminals is also pos-  
sible from a tubular system. Loss of electrondense material from the  
granules and transformation into vesicles marks the release process.

Fridberg *et al.* (1966) studied the regeneration of the caudal neuro-  
secretory system in the cichlid teleost *Tilapia mossambica*. Prominent



tubular reticula were noted in the distal parts (preterminal) of the neurosecretory axons and evidences for local axonal production of vesicles and granules were there. Signs of secretion into the central canal were noted.

Microtubules and mitochondria have been noted in the neurosecretory axons. Axoplasmic reticulum is formed by invagination of axon membranes and cisternae thus formed will contain low electron density-material. In this way elementary neurosecretory granules are formed both in the axons and in the preterminal regions. Kobayashi *et al.* (1963), Uemura *et al.* (1963) and Oota (1963a, b) think that the small vesicles are synaptic and they contain acetylcholine. Baumgarten and Wartenberg (1970) noted adrenergic neurones in the lower-spinal cord of the pike (*Esox lucius*) and studied their relation to the neurosecretory system of the neurophysis spinalis caudalis. Regarding the function of the adrenergic innervation of the neurosecretory system they think "that the short local adrenergic neurones in the lower spinal cord of teleosts antagonize the hormone releasing effects of the cholinergic excitatory influence, *i.e.*, inhibit the release of neurosecretory material."

#### METHODS OF DISCHARGE OF NEUROSECRETORY MATERIAL

1. Release of intact elementary granules into basement membrane region.
2. Diffusion of secretion from the membrane-limited granule.
3. Fragmentation of granule envelope into synaptic like small vesicles. These vesicles fuse with the axon membrane and release takes place by exocytic process.
4. Small vesicles have been noted in the basement membrane region.
5. Depolarization of the terminal membrane facilitates release.  $K^+$  and  $Ca^{++}$  help release of the material.
6. Extrusion of the granules into the central canal of the caudal spinal cord. Disintegration of the elementary granules within the cell may also help release.

Speidel (1919) described gland-cells of internal secretion in the spinal cord of skates. The cytoplasm of these big cells was homogeneous at rest. It contained chromophilic substance. Small vacuoles appeared in the cytoplasm and a big vacuole formed by the coalescence of the smaller ones. With the formation of the vacuole there was no material in it and in a more advanced stage the large vacuoles are peripherally situated with a slight acid-staining precipitate in them. Granules grow in numbers and in size.

The *gland-cell hypothesis* of Speidel (1919) assumes "that these peculiar cells of the skate are transformed nerve-tissue." The granules are formed in the vacuoles and are discharged toward the ventral side of the spinal cord due to the movements of the neurolymph. Discharge took place in a network of blood vessels just ventral to the central canal. Here most of the granules were probably absorbed. The granules were of protein nature.



Speidel concludes "The evidence, morphological and experimental, indicates that the cells are gland-cells of internal secretion. The experimental evidence consists in increase in volume of granular material following electrical and pilocarpine stimulation of the spinal cord. No increase in volume of granular material follows atropine stimulation."

Degranulation response of the preoptic cells has been noted after electrical stimulation of different parts of the brain and the spinal cord in the teleost, *O. Punctatus* (Roy, 1969). In *Bufo melanostictus* Roy (1970) found loss of nsm from the magnocellular preoptic nuclei, the hypothalamo-hypophysial tract and the neurohypophysis. Reaccumulation started from the terminal end towards the cell body and depended on the time interval between the cessation of stimulation and preparation for examination. With stimulation of retina, loss of nsm was detected as in olfactory tract stimulation ; but the magnitude of response was lesser.

Caudal neurosecretory cells have axo-dendritic, axo-somatic and axo-axonic synapses upon them (Ishibashi, 1962 ; Fridberg, 1963 ; Bern *et al.*, 1965 ; Fridberg *et al.*, 1966 ; Sano *et al.*, 1966). These neurones can conduct impulses in teleosts (Morita *et al.*, 1961 ; Bennett and Fox, 1962 ; Yagi and Bern, 1963, 1965). Similar feature has been observed by Bennett and Fox (1962) in elasmobranchs. Prolonged action potential is a characteristic feature. Fridberg *et al.* (1966) noted the relation of impulse conduction to electrically induced release of neurosecretory material from the urophysis of the teleost fish, *Tilapia mossambica*. After intense and prolonged electrical stimulation, repeated firing of neurosecretory units was noted in the *in vivo* preparations. Compound action potentials were recorded *in vitro*. On stimulation there was depletion of nsm from the urophysis.

Increased frequency of spontaneous discharges of urophysial system was noted by Yagi and Bern (1963) in fresh water *Tiapia* after exposure of their gills to saline. Tap water exposure had opposite effect.

Synaptic activation of the neurosecretory neurones was noted after stimulation of the forepart of the spinal cord. The presynaptic fibres were of high threshold and the conduction velocity was low.

Neurosecretory units in *Tilapia* were classified into one minor and two major types according to the response obtained after changing osmolarity of blood (Yagi and Bern, 1963, 1965). The major types included those where (a) increase in discharge rate was noted after intravenous infusion of hypotonic NaCl solution, and (b) activation occurred after infusion of hypertonic NaCl solution. No response to osmotic stimuli was noted in the third/minor type of neurosecretory unit. Sodium ions of the blood were responsible for this response and changes in osmotic pressure were not at all responsible.

Fridberg and Bern (1958) proposed a scheme representing the role of urophysis in osmoregulation. There is a control centre ( $\text{Na}^+$ ) in the brain. Presynaptic fibres make synaptic connections with Dahlgren cells in the spinal cord. Urophysial hormone/s are liberated into renal portal system. There is renal diuresis and increased  $\text{Na}^+$  retention (in the eel



however,  $\text{Na}^+$  excretion happens). In the gills there is increased  $\text{Na}^+$  influx.

Lederis (1970) summarized the effects of urophysial preparations in Table 1 (page 466). Urophysial Laboratory Standard preparation is known as UHS. The general name given to it is *Urotensin*. "A unit of activity was defined as the trout bladder—contracting (or equivalent) activity present in an extract of 1 mg. of acetone dried urophysis powder of *Gillichthys mirabilis* homogenized in 0.25% acetic acid and heated for 3 min. in a boiling water bath." Sawyer and Bern (1963) did not find substances in the teleost urophysis having characteristics of neurohypophysial hormone peptides. Sterba *et al.* (1965) however, found substances like arginine vasotocin and isotocin. The putative carrier protein has been called *urophysin* by Lederis and Bern.

Lederis (1970) thought of the existence of three vasoactive substances in the urophysis. Urotensin has effects on the smooth muscle of the ovary of *Lebistes reticulatus*. "It seems likely that the bladder-contracting activity, and one or more of the vasoactive principles, may be peptidic in nature and of a relatively low molecular weight (mol. wt. 1,000 or less)."

There is uncertainty at present (Bern, 1971) whether the substances showing pressor activity in Chan-Chester Jones studies, showing depressor activity in Kobayashi-Matsui-Hirano-Iwata-Ishii studies, and showing smooth muscle-contracting activity in Lederis, are identical. Kobayashi, Yasumasu and Matsui could isolate two substances with vasodepressor activity in the rat from carp urophysis. One factor is possibly a polypeptide (acute effect) and the other possibly contains sugars and amino-acids (long lasting depressor action).

The urophysial active substances are polypeptides and these can be distinguished from other peptide hormones, *e.g.*, neurohypophysial peptides, angiotensin II and bradykinin (Chan and Chester Jones, 1967, 1969; Chan, 1969; Chan and Ho, 1969; Chester Jones, Chan and Rankin, 1969).

Chan (1971) stressed importance on the caudal circulation and the urophysis of teleost fish. The caudal circulation has been described in the Asiatic eel. The teleost fish has a lymphatic system and a lymph heart with two chambers. Lymph enters into the right-sided chamber of the lymph heart and it is pumped into the caudal vein by the left-sided chamber. The Cerebrospinal fluid joins the lymphatic system. (Figs. 25, 26 from Grasse, 1958, page 1450). Lymphatic system and caudal lymph heart are also present in the cyclostomes (*Myxine glutinosa*) (Fig. 27 from Grasse 1958, page 96).

Chan (1971) noted increase of blood pressure in the caudal vein and increase of pulse rate of the caudal lymph heart of eel after injection of extracts of urophysis from the eel and horse mackerel. The caudal heart rate was increased when blood was withdrawn from eels. The response was found to be abolished after division of the urophysial tract. Less active material was found from urophysis of bled animals. The caudal neurosecretory neurones and terminals from bled animals had less stainable material when they were compared with controls. Dose-dependent



elevation of caudal heart rate was found after 5-Hydroxytryptamine. "The effect of 5-HT but not of urophysial extract was diminished by bromo-LSD and completely abolished by transection of the urophysial tract." In the eel, material carried in the cerebrospinal fluid enters the lymph heart through the lymphatics and nsm discharged in the C. S. fluid may act on the caudal lymph heart. The tip of the caudal vein is pulsatile in those teleostean species where the lymph heart is absent (In Grasse, 1958). Thus the caudal neurosecretory system may be responsible for the control of flow of the body fluid from the posterior part of the body and adequate blood pressure is maintained in the renal portal system (Chan, 1971). Chan also agreed that the lymph heart stimulating substance (LHSS) is different from urotensins.

Lederis *et al.* (1971) thought of two active principles from urophysis. One is Lebistes ovary-oviduct-stimulating principle and the other is involved in trout bladder, fish-intestine contraction and hypertensive in the eel. They are peptidic in nature.

Vigh-Teichmann and Vigh (1970) studied the structure and function of the liquor contacting neurosecretory system. Small, bipolar neurones having a strong acetylcholinesterase activity in dehydrated fishes were noted around the central canal, in the region of the urophysis. The arrangement of the cells was in one ventral and two lateral groups. One bulblike process showing AchE activity was noted in the lumen of the central canal. Such liquor contacting neurones were found in other segments of the central canal of the spinal cord.

Professor Bern (1971) stated in Chairman's opening remarks that the urophysis is an endocrine organ and there are some unanswered questions. He states "Our knowledge of its function, in an organismal-physiological sense, is virtually nil and the system remains a challenge to the comparative endocrinologist and the fish physiologist."

### *Phylogeny of neurosecretory cells.*

Gabe (1966) discussed this topic and mentioned about two views. According to one view ordinary neurones appear first and by progressive differentiation the neurosecretory cell is evolved. The neurosecretory cell is thus a modified neurone. The other view suggests that neurosecretory cells may have significance of epidermal gland cells and they are secondarily incorporated in nerve centres. Gabe supported Scharrer's concept making the neurosecretion a connecting link between the nervous system and endocrine glands and further thinks that "neurosecretion plays an essential part in maintaining equilibrium between the organism and its surroundings."

Glandular and neural elements are derived from undifferentiated cells with basic properties of excitability, conductivity and formation of physiologically active substances. In both these elements secretory activity is noted but the degree of this activity is variable depending on the structural and functional specialization. Ordinary neurones have digressed from the ancestral pattern more than the neurosecretory cells. Scharrer and



Weitzman (1970) further state that "the questions to be considered then are merely how far back the dichotomy occurred, and where the dividing line between the neurosecretory and conventional neurones should be drawn."

*Caudal part from lower to higher fishes (Figs. 29, 30, 31, 32)*

There are no nervous elements in the caudal part of the spinal cord of *Amphioxus*. Only the ependymal tube is present with terminal ampullar enlargement. The large cells are oriented in such a fashion that their inner surfaces look backwards (Ariens Kappers *et al.*, 1967).

In the caudal part of *Petromyzon* spinal cord there is no nervous tissue. There is terminal caudal enlargement of the central canal. Surrounding the central canal of this region there are plenty polynuclear cells with glandular activities.

In the seahorse (Syngnathidae) and the ocean sun-fish (Molidae) the caudal neurosecretory system is absent. Chan (1971) thinks that the tail fin and musculature are reduced to a great extent in these two animals and they do not take part in locomotor functions.

Lobate nature of the urophysis is absent (Fig. 28) in Dipnoans having *protocercal* type of tail fin, in some elasmobranches having *heterocercal* type of tail fin and in *Amia* having *hemihomocercal* type of tail fin. Teleosts having *homocercal* type of tail fin have usually a lobate urophysis. One fact is very clear that in order to have a distinct urophysis, the last vertebral body (urostyle) must have a depression for accommodating the same.

### CONCLUSION

Progress in the study of caudal neurosecretory system of fishes since the time of Enami has been discussed. Ultra-structure and physiology of the same system have been elaborated further through these works. The idea of activation of caudal neurosecretory cells by nervous and humoral stimuli as enunciated by Roy (1962) has been largely substantiated in the subsequent years. The recent works also tend to substantiate the observation of Roy (1962) regarding the peptidic nature of the hormone elaborated by the caudal neurosecretory cells.

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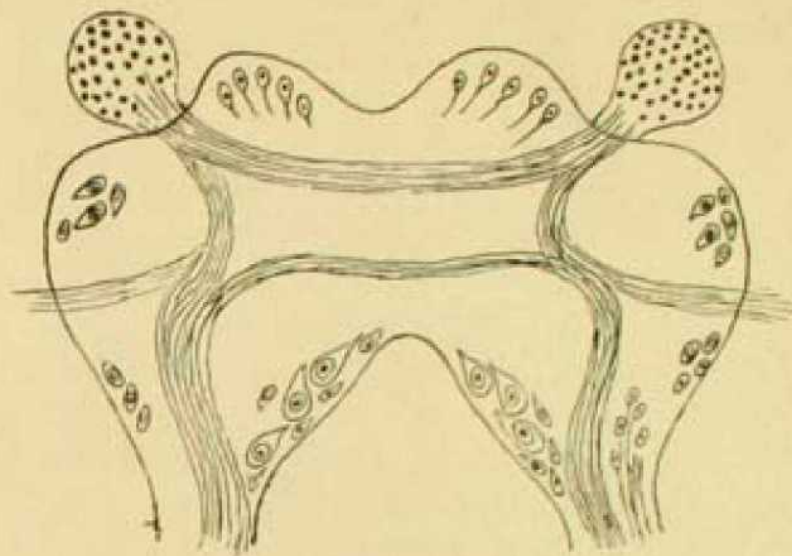
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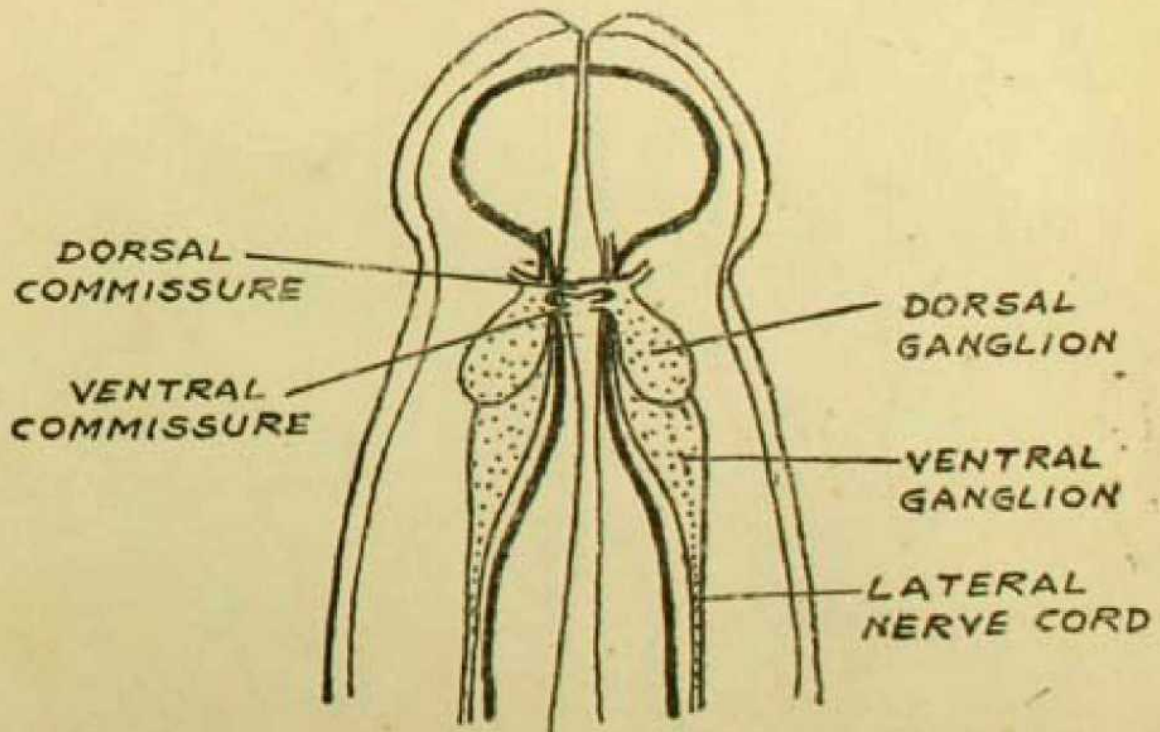


## Chapter 14



**T.S. OF CEREBRAL GANGLION OF THE  
POLYCLAD TURBELLARIAN NOTOPLANA**  
*Fig. 1*

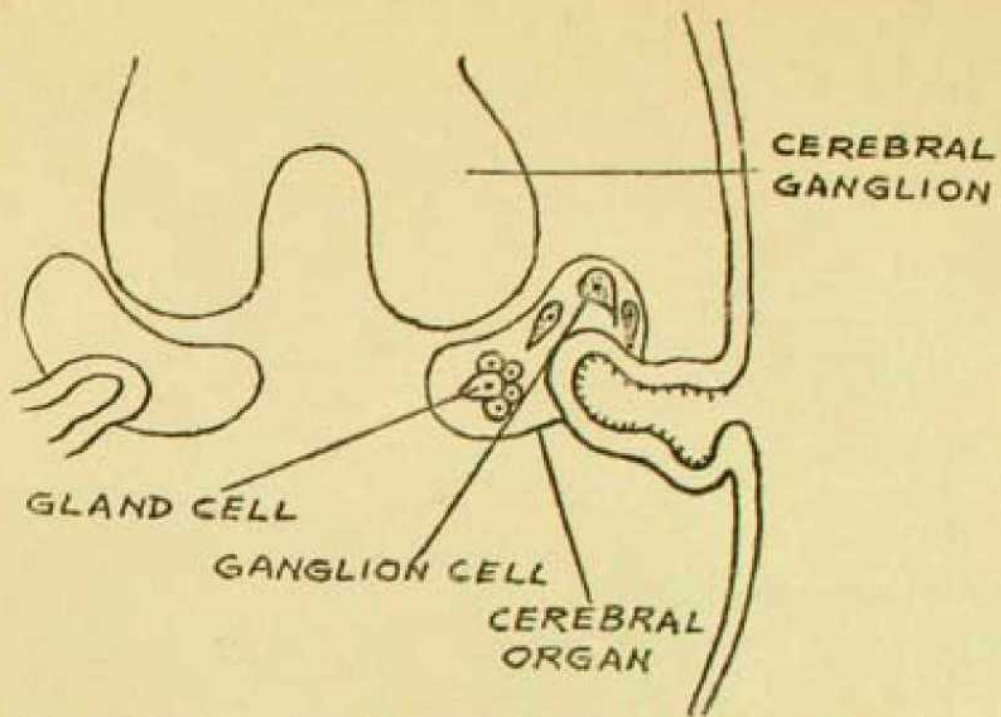
Transverse section of the cerebral ganglion of the polyclad turbellarian Notoplana showing the different types of cells. (After Hadenfeldt).



**FOREPART OF A NEMERTEAN  
SHOWING GANGLIONIC MASSES**  
*Fig. 2*

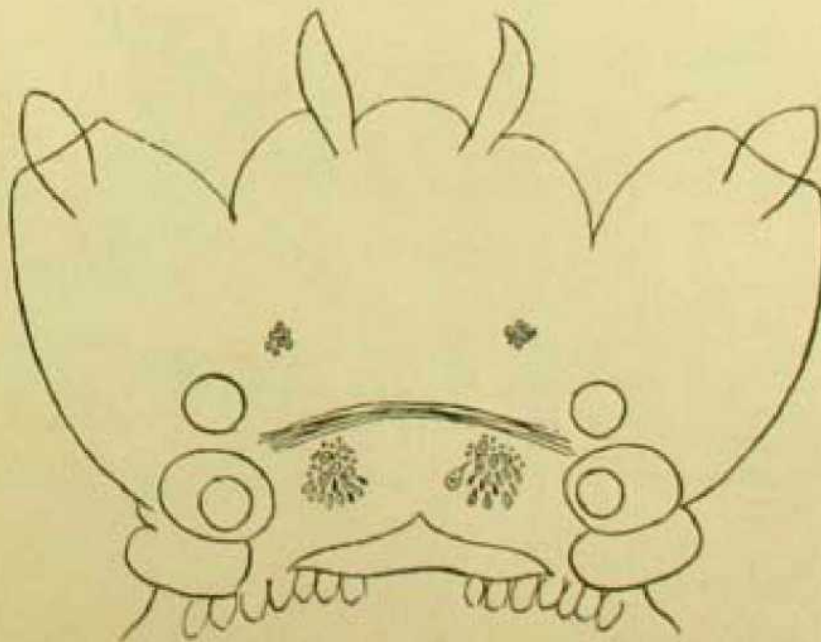
Anterior part of a nemertean showing ganglionic masses.





**CEREBRAL ORGAN OF A NEMERTEAN**  
*Fig. 3*

The neuroglandular cerebral organ of nemertean.

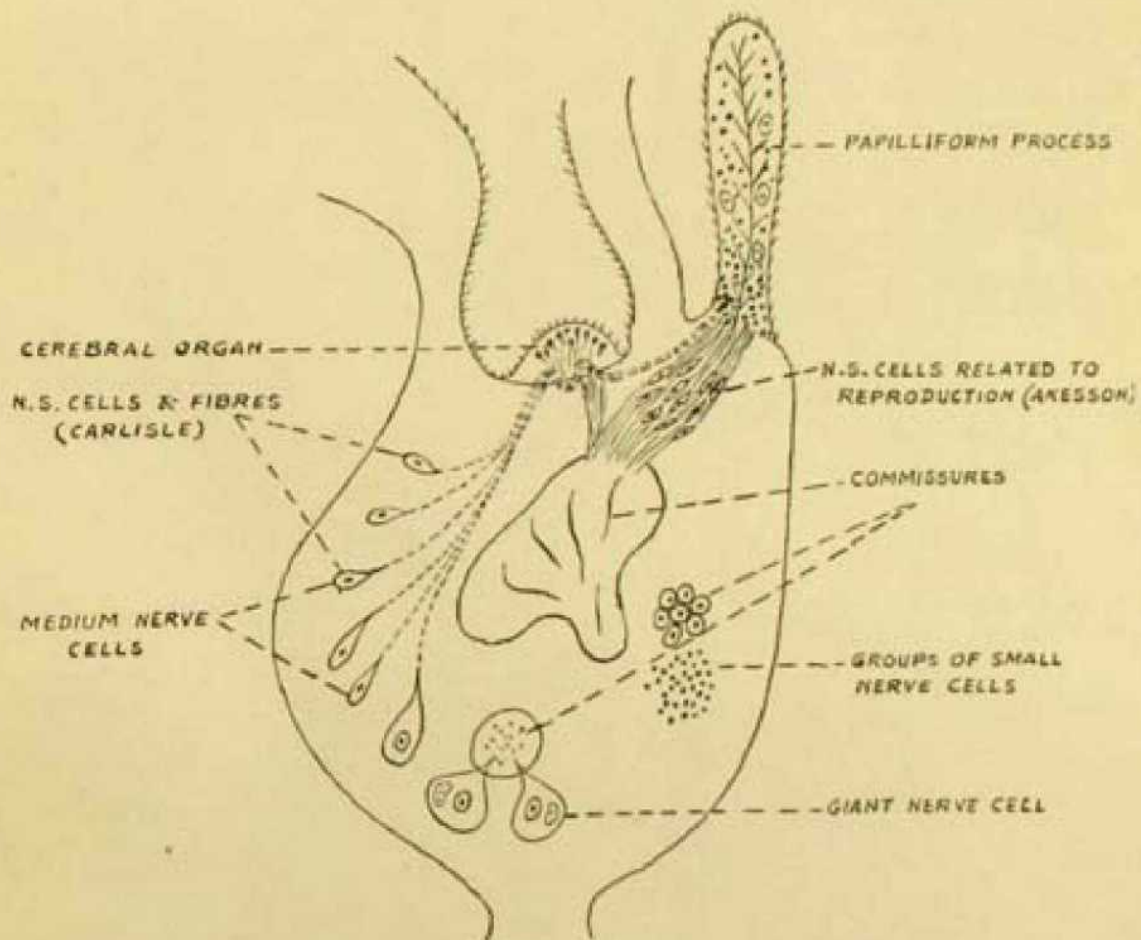


**N.S. CELLS IN THE BRAIN OF  
 NEREIS DIVERSICOLOR**

*Fig. 4*

Shows the neurosecretory cells in the brain of *Nereis diversicolor*.

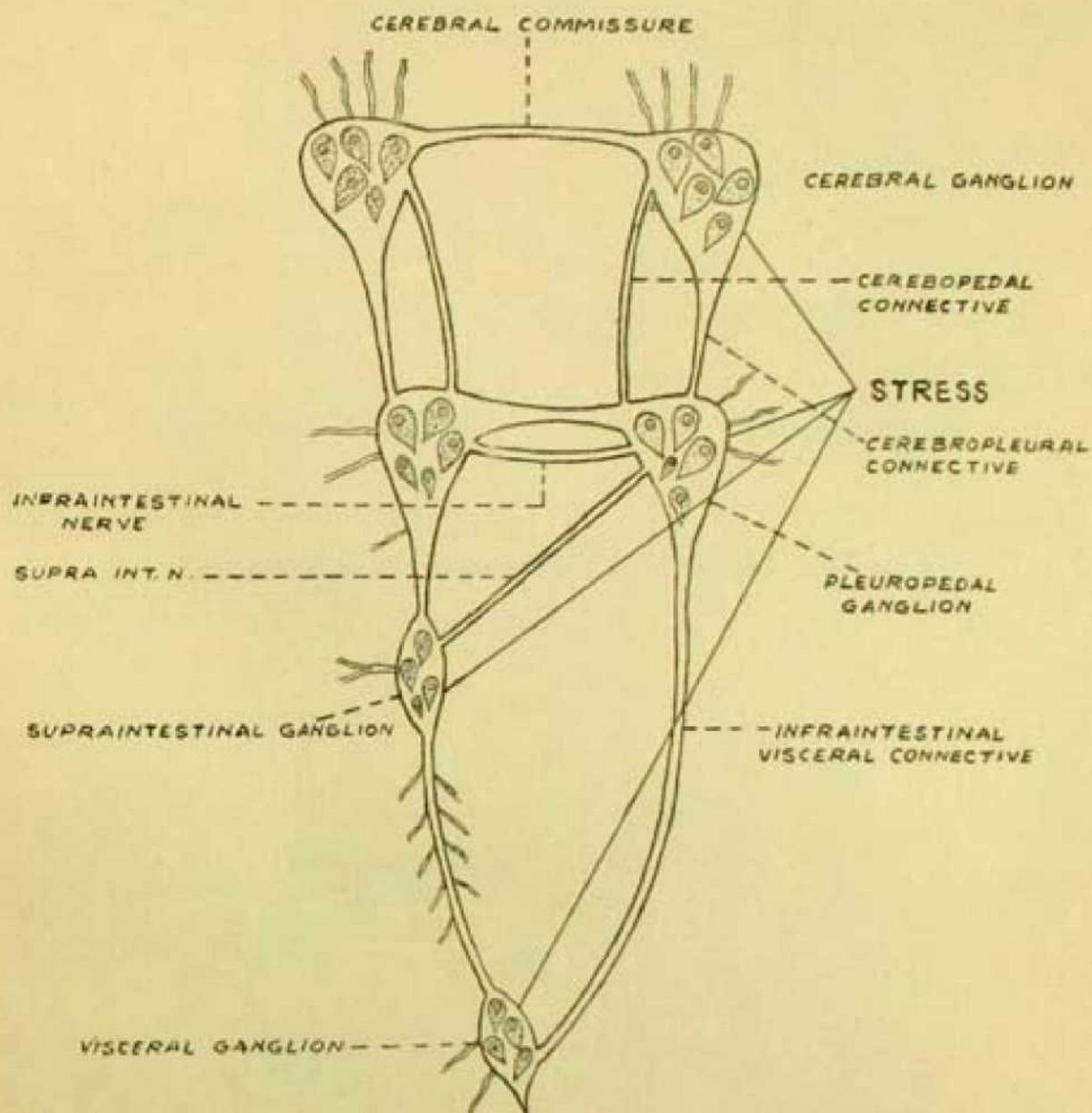




**BRAIN OF SIPUNCULUS NUDUS**  
**WITH N.S. CELLS AND ENDORGAN**  
*Fig. 5*

Brain of *Sipunculus nudus* showing neurosecretory cells and a papilliform process (endorgan).



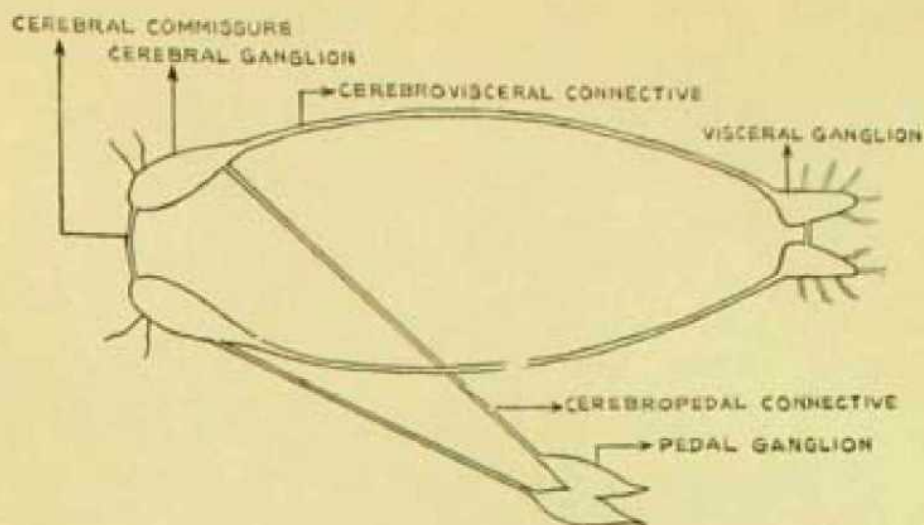


### STRESS AND NEUROSECRETION IN PILA GLOBOSA

Fig. 6

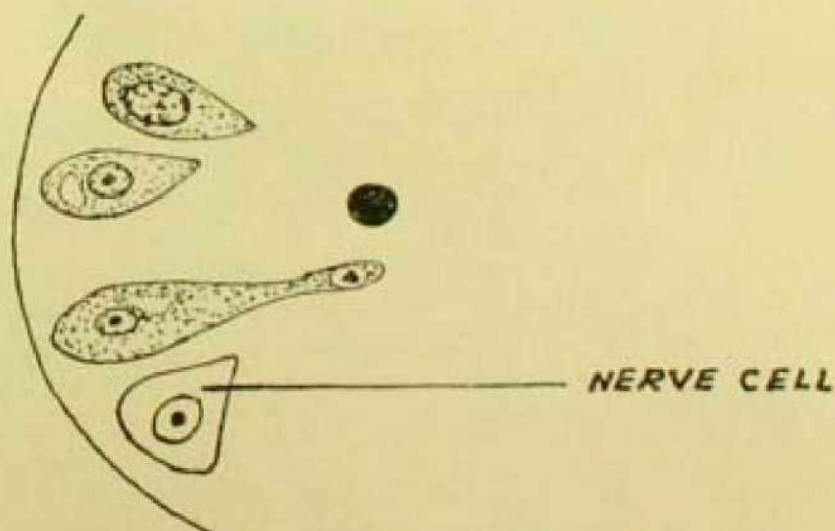
The ganglionic masses and connections in *Pila globosa* showing the effects of stress on neurosecretion.





**NERVOUS SYSTEM OF *LAPELLIDENS MARGINALIS***  
**(SCHEMATIC)**  
**Fig. 6 A**

The ganglionic masses and connections in *Lamellidens marginalis*



**TYPES OF NEUROSECRETORY**  
**CELLS (CEREBRAL GANGLION)**  
**Fig. 7**

Types ( I & II ) of neurosecretory cells in the cerebral ganglion of *Lamellidens marginalis* with a nerve cell. There is a neurosecretory droplet very near a type I cell.



## CHAPTER—15

### THE FOREBRAIN OF VERTEBRATES

1975

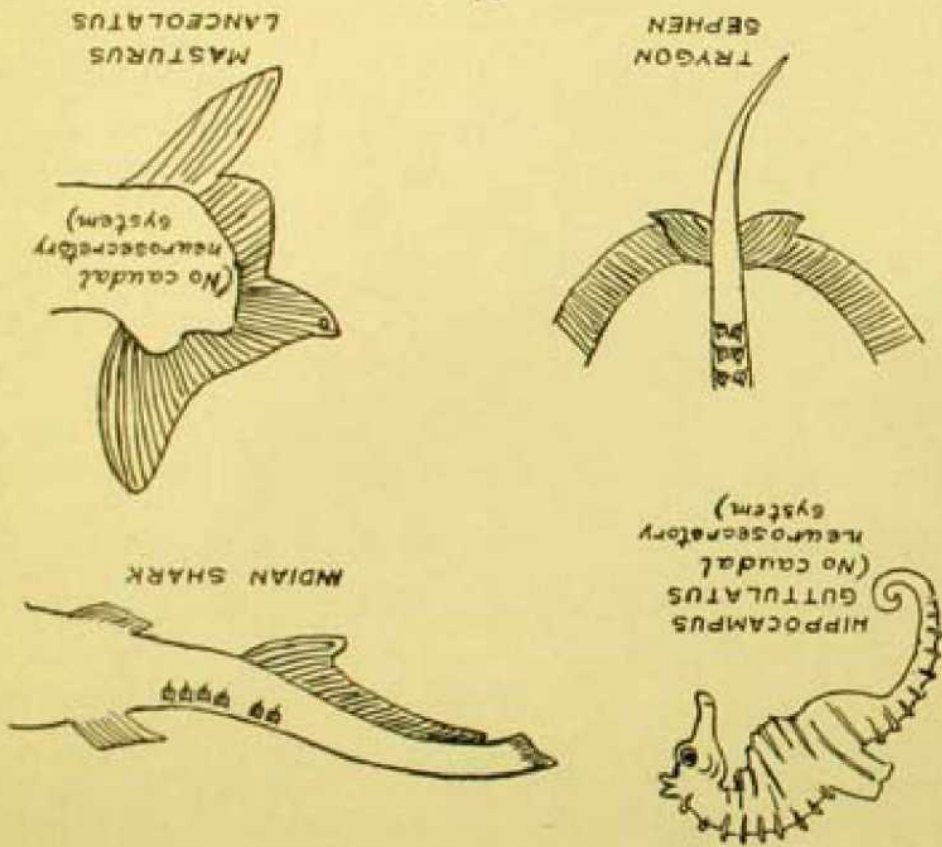
Ariens Kappers (1921) described the various forms of the forebrain which could take place (Figs. R1 to R9). In nearly all animals the pars medioventralis or septum is formed by the medial growth of the pars lateroventralis or striatum. Animals having this type of brain get one large unpaired ventricle and steep lateral walls and a small septum. More differentiated forms also take place. In petromyzonts the mediodorsal portion of the brain belonging to the mantle portion is formed by the bending around of the upper end of the plate in a medial direction. In amphibians there is increase of the pars mediodorsalis which abuts against the large pars medioventralis or septum. In the reptilia there are mediodorsal pallium, laterodorsal pallium, striatum and septum. In the holocephalian chimaera the pars laterodorsalis is small and thickened in the more caudal portion of the forebrain. No pars mediodorsalis is formed from it. The pars lateroventralis or striatum and the pars medioventralis or septum are well developed and much thickened. In ganoids and teleosts the pars laterodorsalis has lesser surface development in comparison to holocaphali with greater thickening. The pars medioventralis (septum) and the pars lateroventralis are well developed. *Everted type* of mantle portion is formed by the greater thickness of the ventriculally directed laterodorsal parts and thus the mantle portion is turned laterally and downwards.

Ariens Kappers *et al.* (1967) reviewed the phylogeny of the telencephalon. The *inverted pallium* is noted in cyclostomes, selachians and all higher vertebrates. The holocephali are regarded by Ariens Kappers to represent a transitional stage between the inverted and everted types. The anterior portion of the mantle is inverted and the exversion is noted only in the caudal portion of the telencephalon and this extends out into the forebrain peduncle. "Other observers (Holmgren) do not regard the Holocephali as intermediate but as related particularly to cyclostomes and selachians." In certain classes of fishes there is an archipallium or primordial hippocampal region in addition to a paleopallial portion. In amphibians there is a general cortical area (forerunner of neopallium) in between the paleopallium and the archipallium. In the reptiles the archicortex is better developed than the paleocortex. The general cortex (neocortex) is better developed in mammals in between paleocortex and archicortex. "In lower mammals the archicortex occupies its primitive position in the midline, but in higher mammals, with the progressive development of the neocortex, the archicortex is rolled backward, lateralward, and ventralward into the temporal pole region, and only traces of it are to be found in the midline region."



Caudal neurosecretory system is absent in *Hippocampus* and *Masturus lanceolatus*. It also shows neurosecretory cells in an Indian shark and *Trygon sephen*. There is no urophysis.

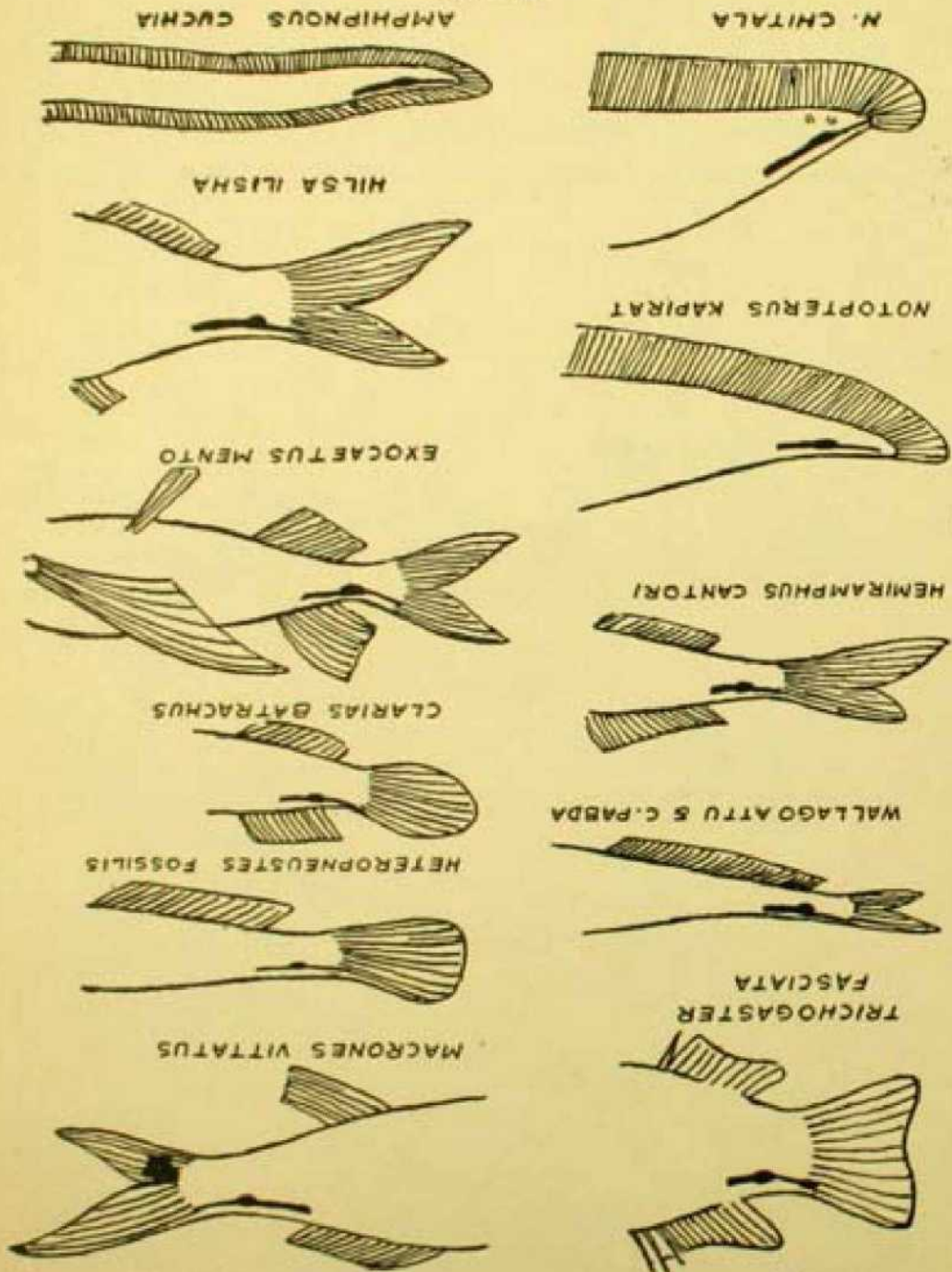
Fig. 32





Caudal parts of different fishes showing position of lobate urophysis.

Fig. 31

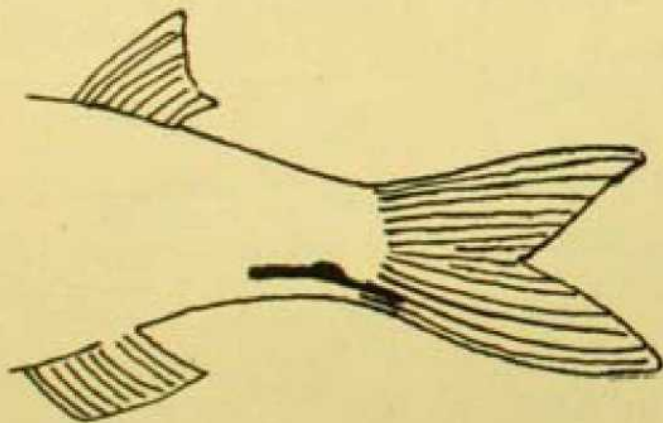




Caudal parts of different fishes showing position of lobate urophysis.

Fig. 30

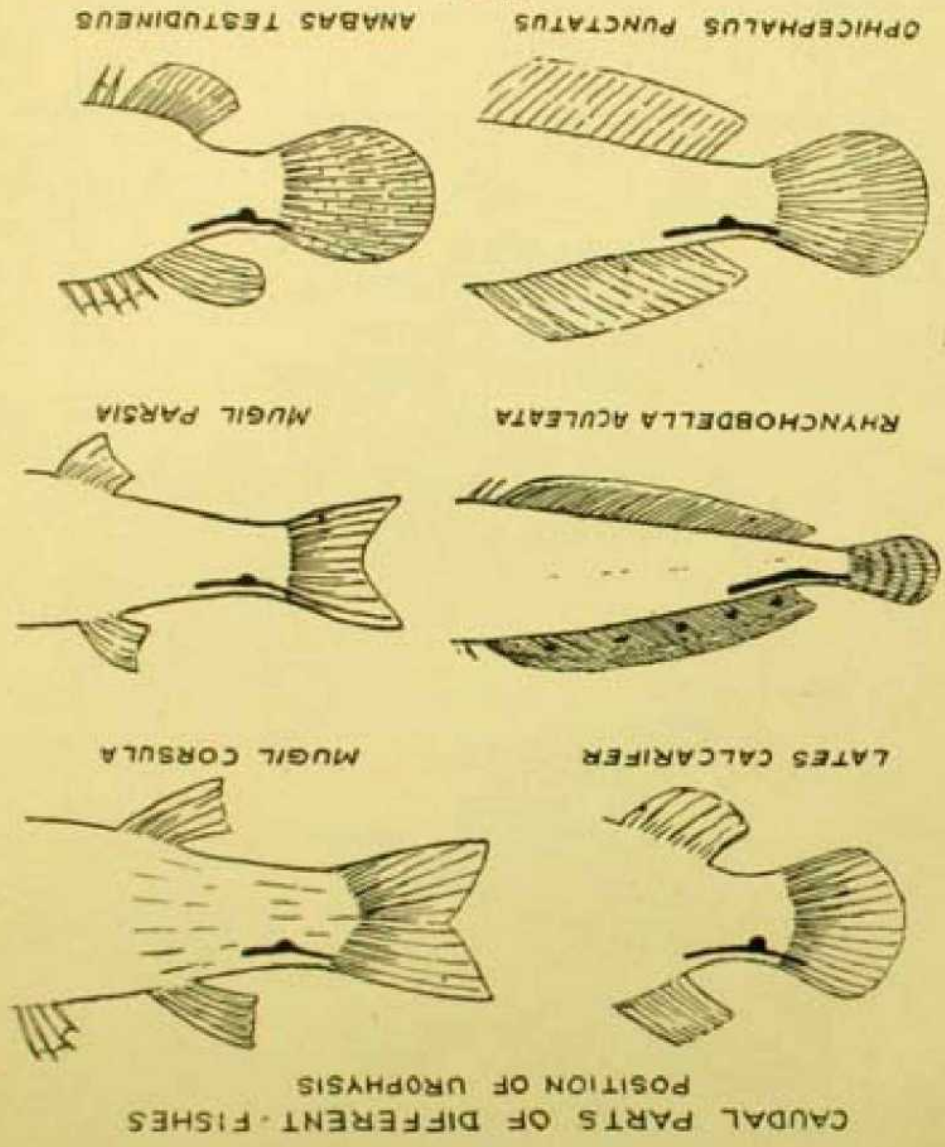
LABEO CALBASU  
 L. BATA  
 L. ROHITA  
 CIRRHINA MRIGALA  
 CATLA CATLA  
 AMBLYPHARYNGODON MOLA  
 BARBUS TICTO





Caudal parts of different fishes showing position of lobate or nonlobate urophysis.

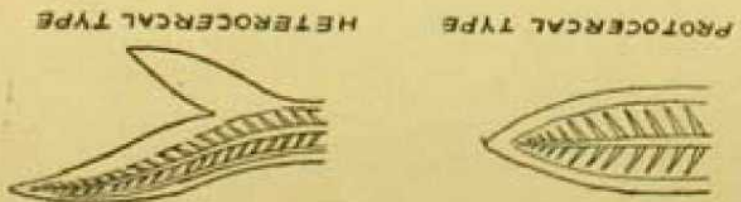
Fig. 29



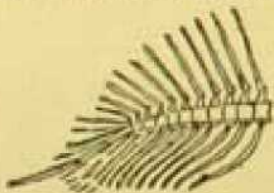
CAUDAL PARTS OF DIFFERENT FISHES  
POSITION OF UROPHYSIS



TYPES OF CAUDAL FINS



HEMIMOMOCERCAL TYPE



HOMOCERCAL TYPE



Types of caudal fins.

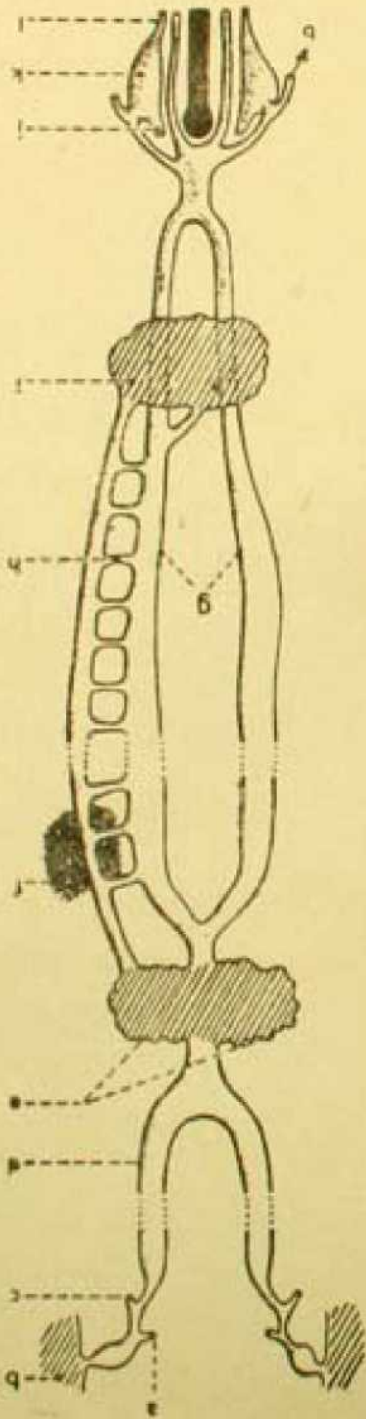
Fig. 28



Lymphatic system of *Myxine glutinosa* with caudal heart (k).  
 —from Grassó

Schéma de l'ensemble  
 du système lymphatique  
 de *Myxine glutinosa*. a, vers  
 le sinus hypopharyngo-velaire;  
 b, sinus sous-cutané; c, vers  
 le sinus coudo-latéral; d,  
 le sinus coudo-latéral; e, post-  
 sinus coudo-latéral; f, partie du  
 tron du cœur; g, plexus capil-  
 laire de la paroi  
 intestinale se vidant dans le  
 tron lymphatique; h, sinus  
 coudo-latéral; i, tronc lyn-  
 phatique intestinal; j, sinus  
 rectal; k, veine efférente du  
 cœur caudal; l, cœur cau-  
 dal; l', veine caudale affé-  
 rente (d'après Coxe).

Fig. 27





Schematic drawing of the caudal lymph heart—from Grasse.

Schéma du cœur lymphatique caudal. — a, atrium; b, ventricule; c, vaisseau cutané latéral; d, vaisseau hémal; e, sinus veineux caudal; f, veine caudale (d'après Favas).

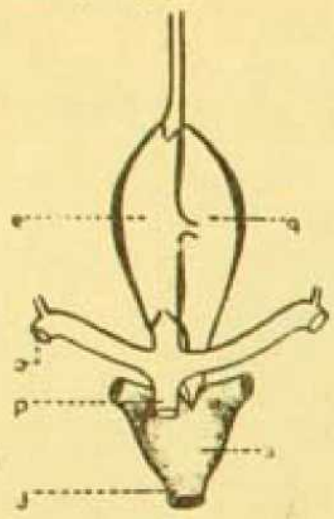


Fig. 26

Vascularization of the caudal part of the eel with caudal lymph heart (d)—from Grasse.

Vascularisation de la nageoire caudale d'une jeune Anguille (Cuvier). — Arteries stiles, veines noires, vertébrales et hypuraux à contour pointillé. — a, aorte caudale; b, veine caudale; c, double arcade artérielle et veineuse qui suit également une arcade lymphatique non figurée; d, molette gauche du cœur lymphatique avec son réseau artériel (le réseau veineux est sur figure de Rouin); e, raccord du cœur lymphatique et de la veine caudale; f, uréostyle; g, h, e, hypuraux (d'après Rouin).

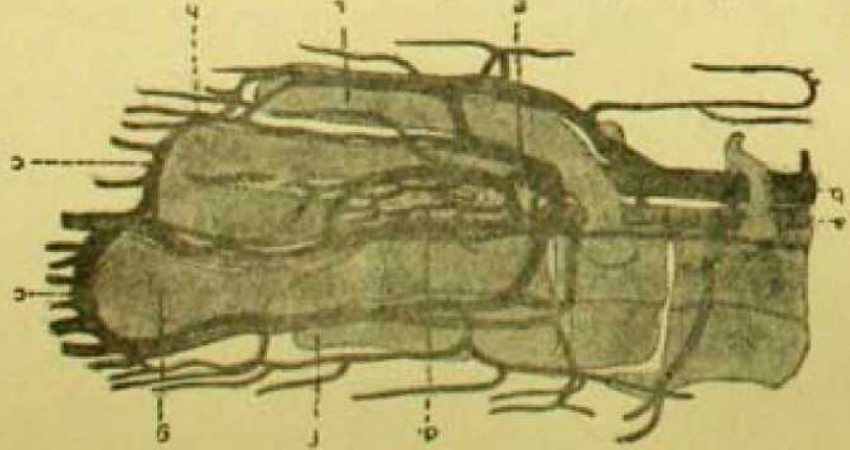


Fig. 25



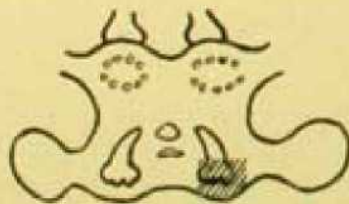
# EFFECTS OF BRAIN LESIONS IN THE GRASSHOPPER

JUMPING ACTIVITY

SOUND PRODUCTION

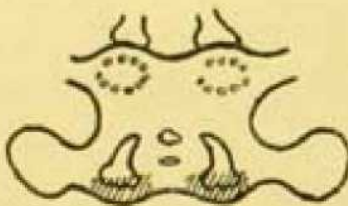
INCREASED (MALE)

NO CHANGE



FURTHER INCREASE

STOPPED



DEPRESSED AFTER  
INITIAL INCREASE

STOPPED

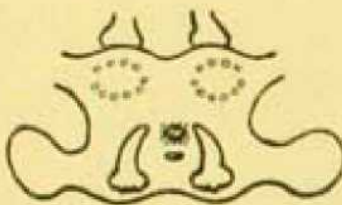


Fig. 24

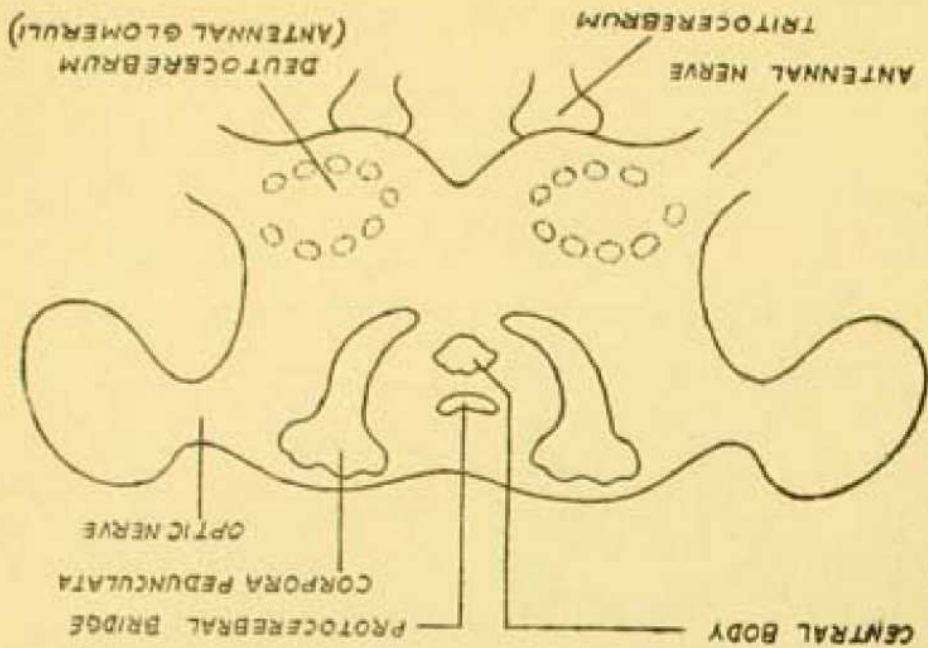
Shows the effects of brain lesion in the grasshopper—after Huber.



Shows the brain of Gryllus—after Huber.

# BRAIN OF GRYLUS (ORTHOPTERA)

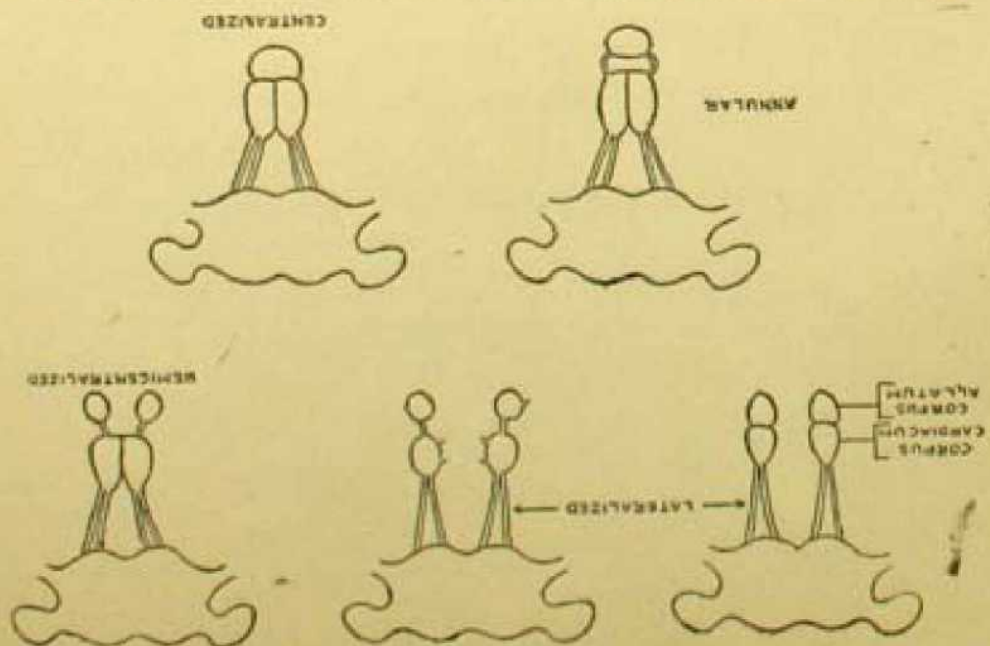
Fig. 23



Types of retrocerebral endocrines in pterygote insects—after Casal.

## TYPES OF RETROCEBREAL ENDOCRINES IN PTERYGOTE INSECTS

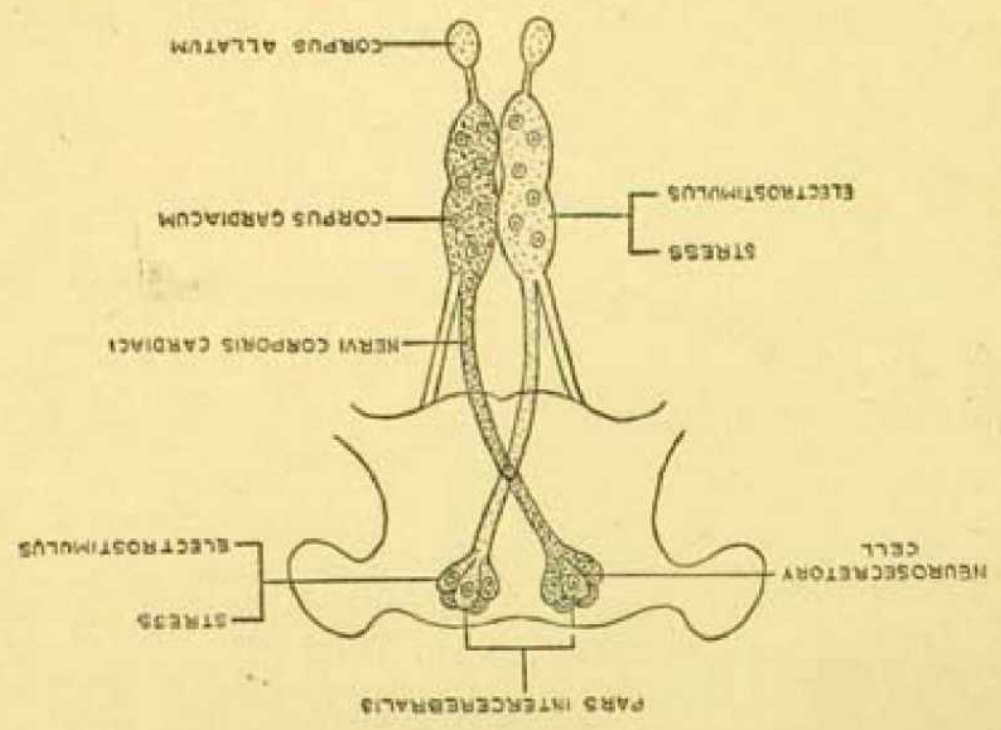
Fig. 22





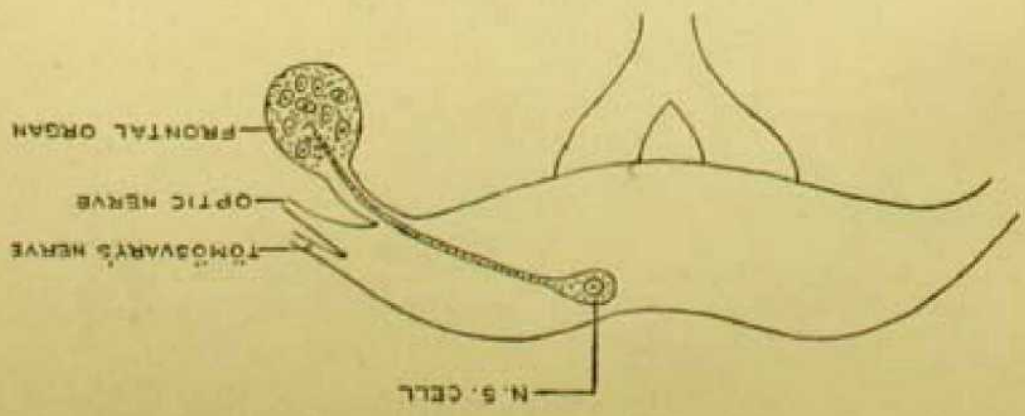
The neurosecretory cell groups in the pars intercerebralis, their axonal paths and end stations in an insect. The diagram also shows the effect of stress and electrostimulus

**STRESS & DEPLETION OF NEUROSECRETION  
IN INSECT**  
FIG. 21



Protocephalic neurosecretory cell in brain of a chilopod Lithobius with neurosecretory path and the cerebral gland.

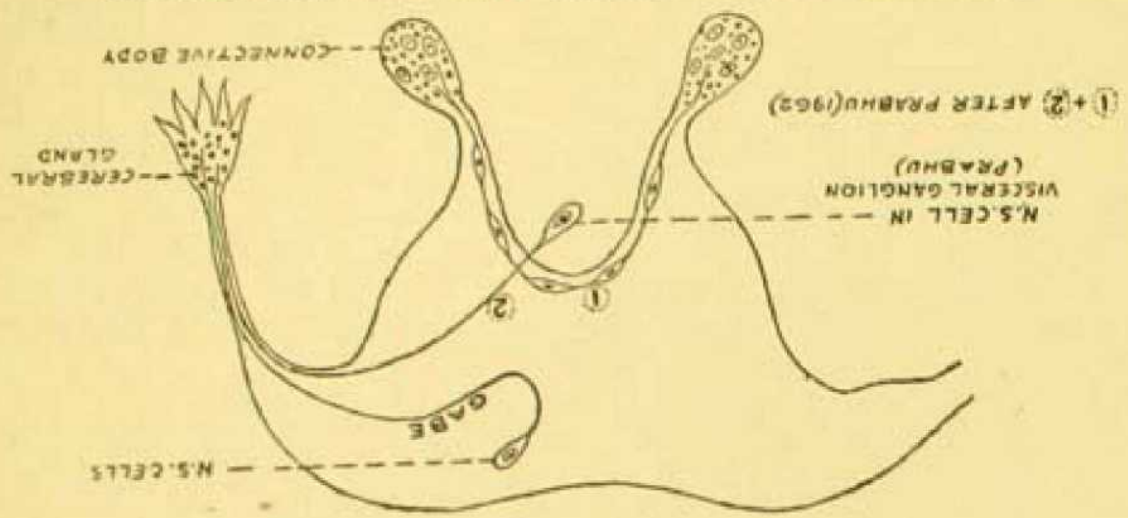
**BRAIN OF CHILOPOD LITHOBIUS**  
FIG. 20





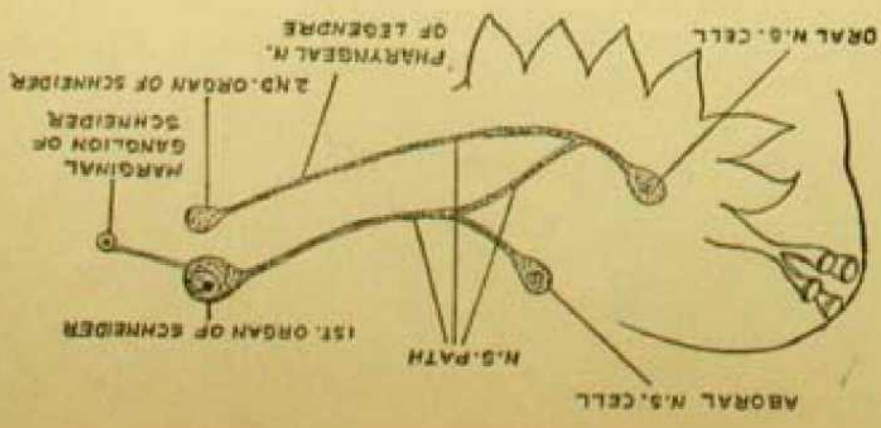
Protocephalic neurosecretory cells in a Diploped brain with neurosecretory paths and end stations

FIG. 19  
DIPLOPOD BRAIN WITH N.S. CELLS AND END STATIONS



Neurosecretory cell groups, the axonal paths and the end organs of a spider.

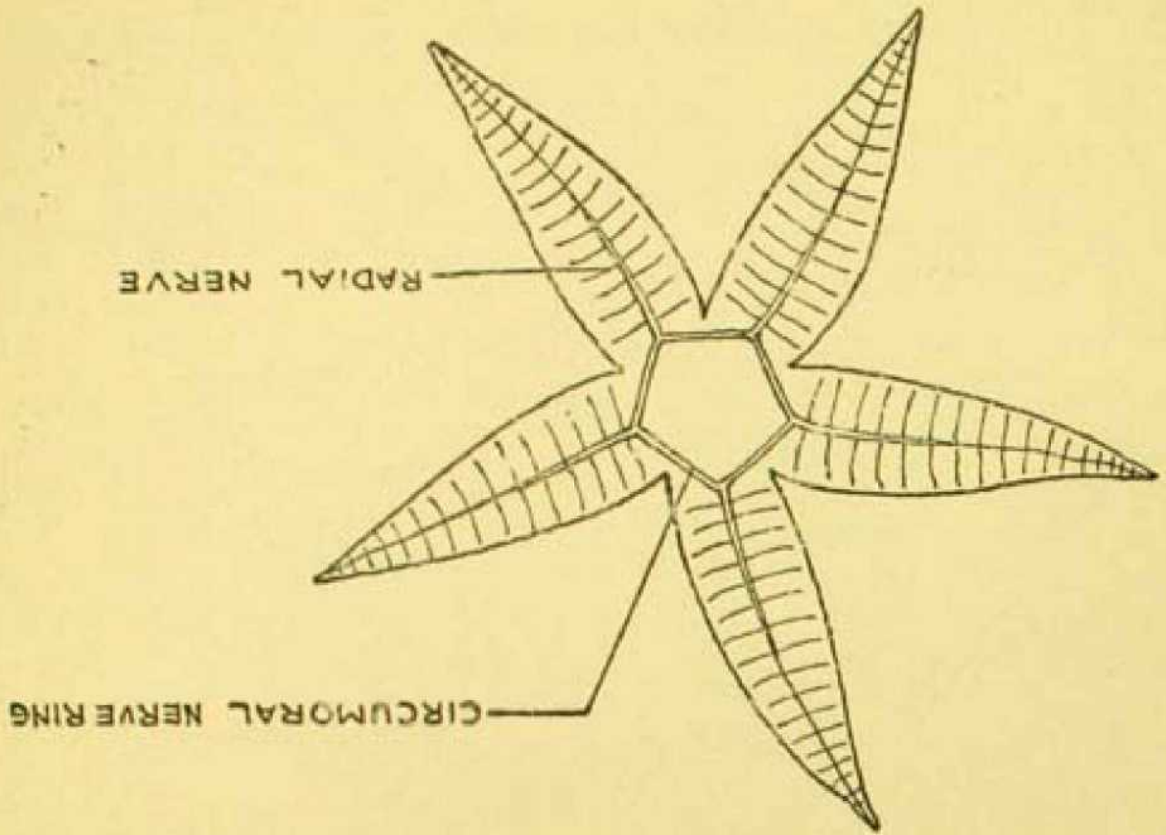
Fig. 18  
NEUROSECRETION IN THE BRAIN OF A SPIDER





# NEUROSECRETION IN A STAR FISH

Fig. 17

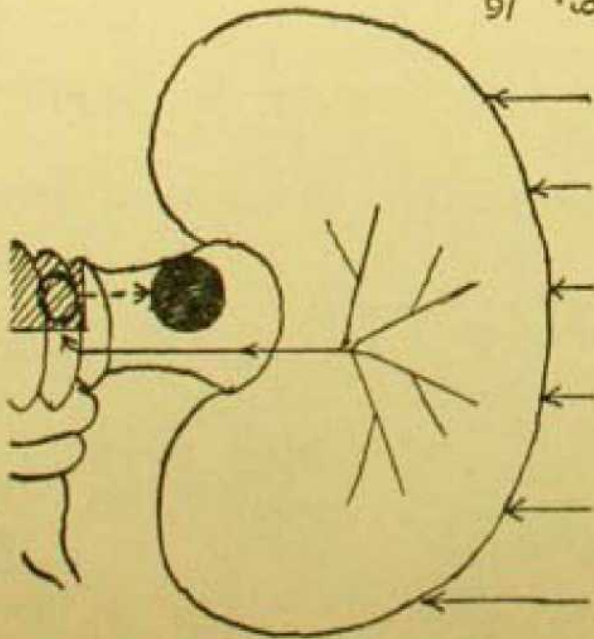


Experiments leading to enlargement of optic gland with maturation of the gonad in octopus,

EXCISION OF SUBPEDUNCULATE LOBE IN VERY YOUNG MALE LEADS TO ENLARGEMENT OF OPTIC GLAND.

EXPERIMENT 5

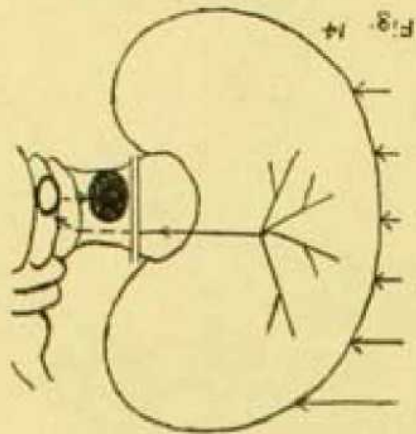
Fig. 16





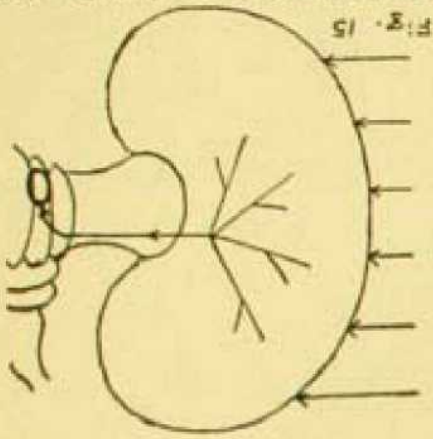
Experiments leading to enlargement of optic gland with maturation of the gonad in octopus.

EXPERIMENT 3  
DIVISION OF OPTIC TRACT  
PROXIMAL TO OPTIC GLAND



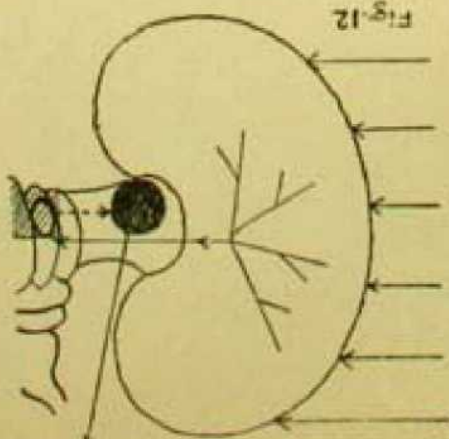
GONADOTROPIC HORMONE +++

EXCISION OF OPTIC GLAND.—NORMAL  
DEVELOPMENT OF OOCYTES. NO  
NORMAL DEVELOPMENT OF FOLLICLE  
CELLS. NO YOLK DEPOSITION.



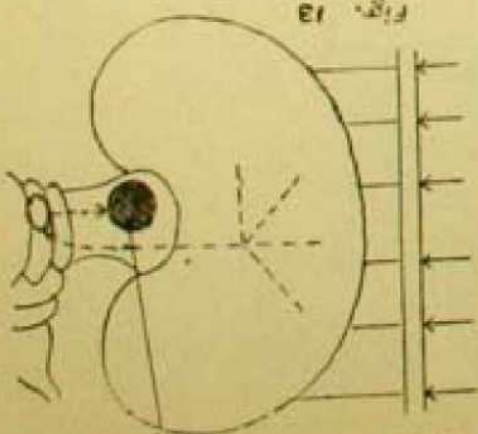
EXPERIMENT 4

EXPERIMENT 1  
EXCISION OF SUBPUDUCULATE  
LOBE



GONADOTROPIC HORMONE +++

EXPERIMENT 2  
DIVISION OF OPTIC NERVES

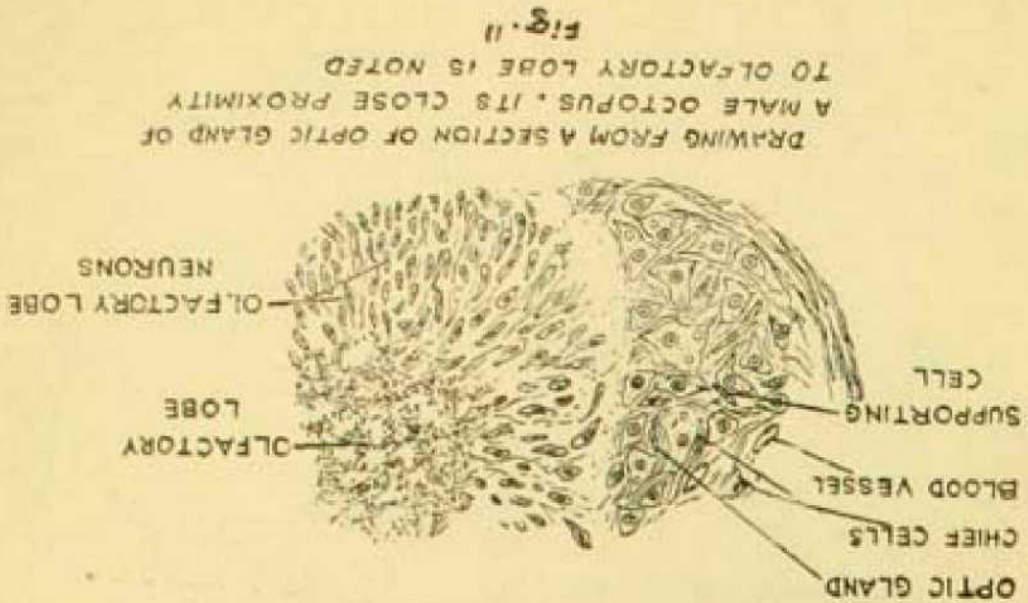


GONADOTROPIC HORMONE +++

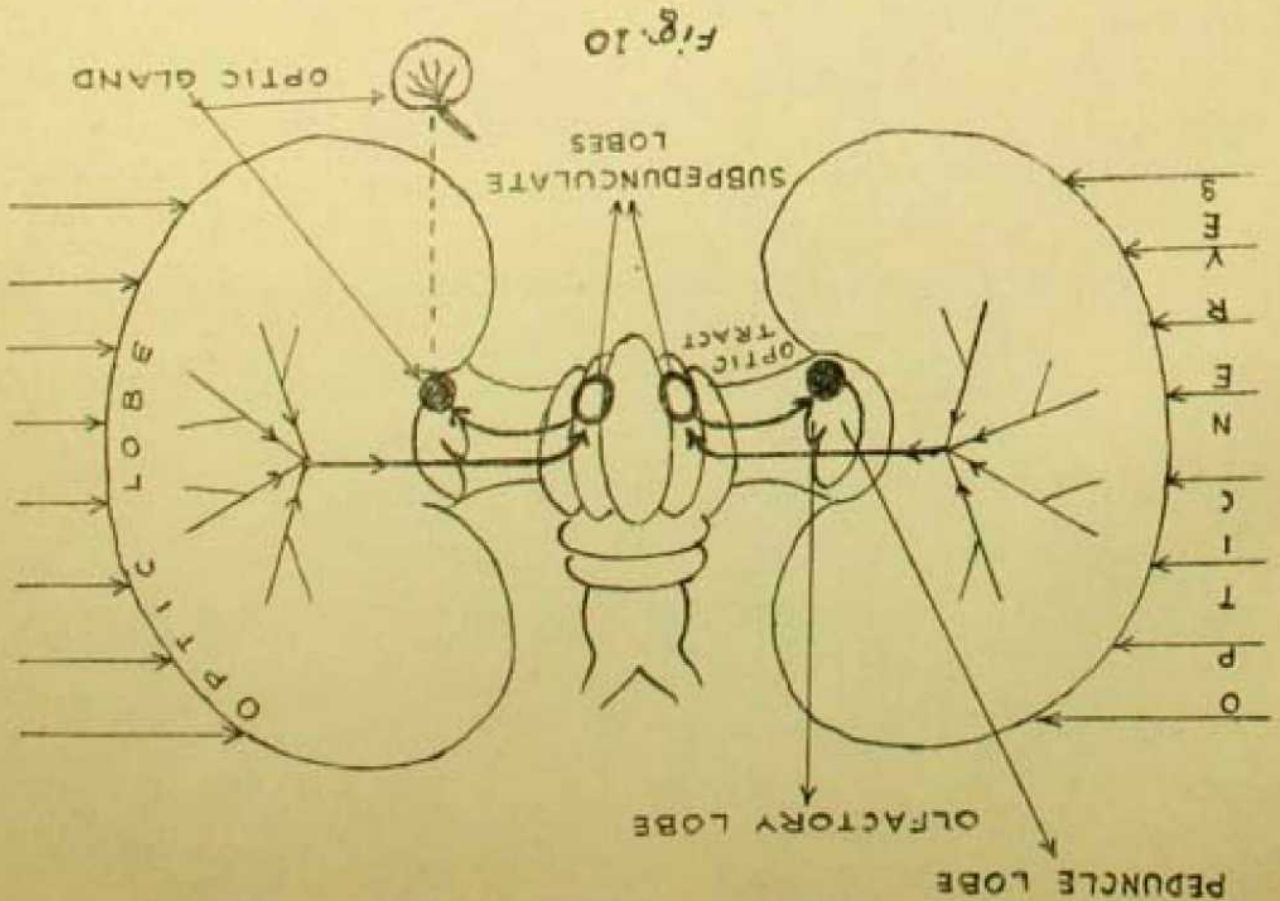
Experiments leading to enlargement of optic gland with maturation of the gonad in octopus.



Drawing from a section of the optic gland of a male octopus. The fine structure of the gland and olfactory ganglion is noted. A very close association of the two structures is evident.



Shows the position of the optic gland and its connection with the subpedunculate lobe of the brain of octopus

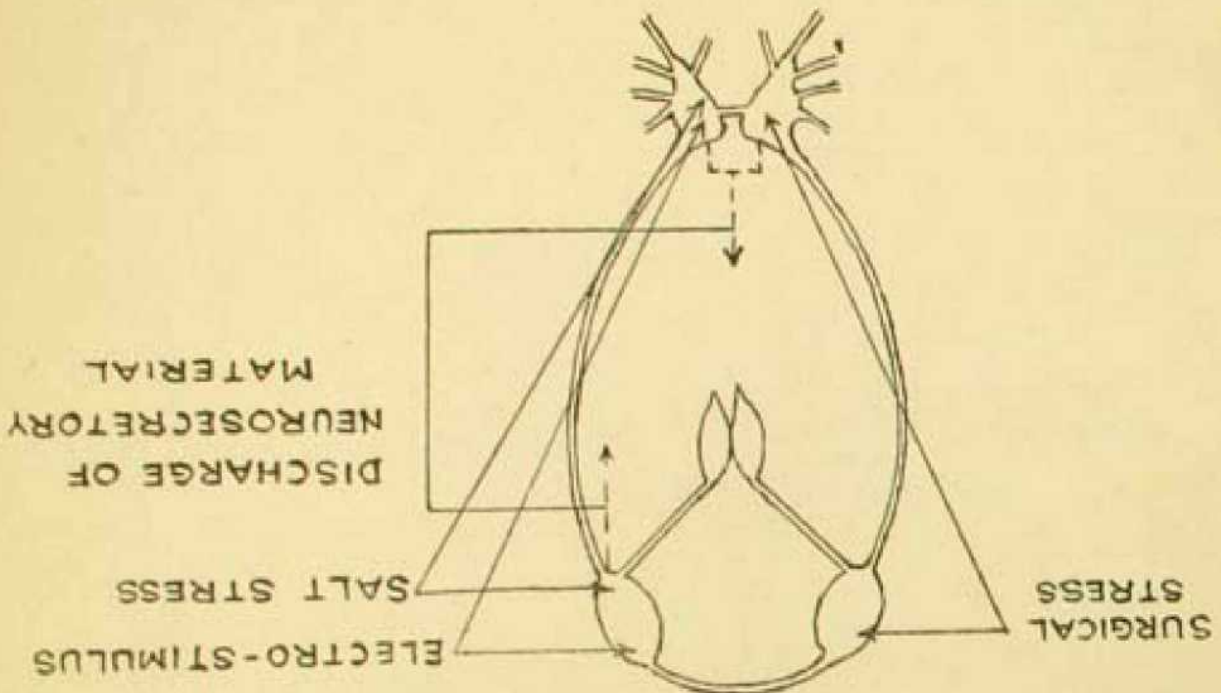




Shows stress and neurosecretion in *Lamellidens marginalis*.

Fig. 9

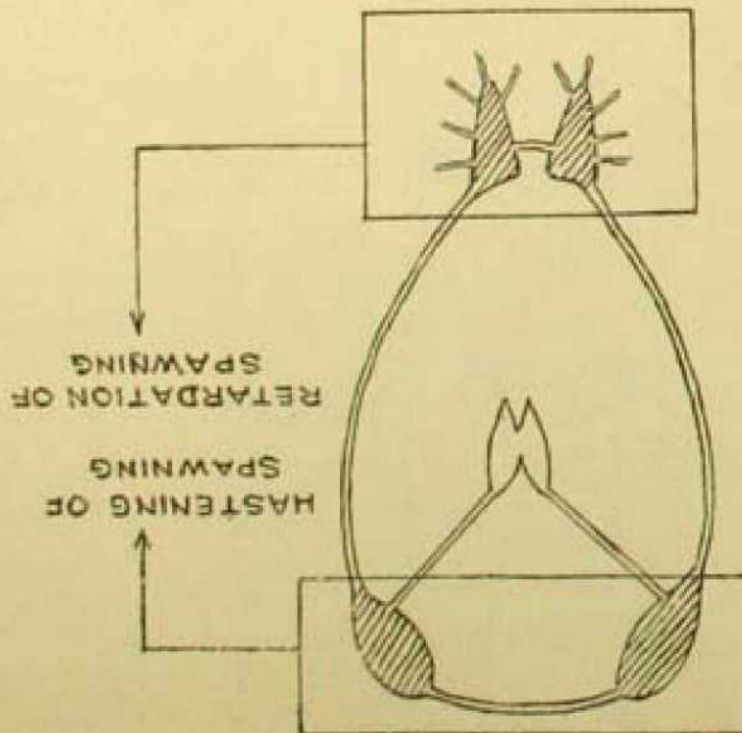
# STRESS AND NEUROSECRETION



Spawning reaction after ablation studies in *Lamellidens marginalis*.

Fig. 8

# ABLATION STUDIES







## MEDIAL FOREBRAIN BUNDLE

*Plagiostomes :*

The tractus medianus connects the archipallial region with the hypothalamic areas. This tract has been considered by some as homologous with the fornix. The most medial fibres of the basal forebrain bundle is the tractus septothalamicus and hypothalamicus or *medial forebrain bundle*.

*Ganoids and teleosts :*

The medial forebrain bundle in teleosts is a connection between the precommissural and septal regions of the telencephalon and hypothalamic areas.

*Amphibians :*

It carries both ascending and descending fibres. It connects the ventromedial portion of the hemisphere to the hypothalamus or lower centres. Anteriorly it is associated with the nucleus olfactorius anterior and the septal nuclei. Primordium hippocampi and primordium pallii dorsalis can be traced more caudally. Primitive fornix system with decussations at the commissura pallii anterior is also noted. This descends to the hypothalamic regions. Decussation of the medial forebrain bundle takes place in the anterior commissure. In between the dorsal and the ventral bundles there are thick fornix fibres.

*Reptiles :*

The medial forebrain bundle forms a large fiber system interconnecting the cortical and basal portions of the medial hemisphere wall with the hypothalamus and tegmental centres. The bundle comprises both ascending and descending systems. The bundle appears in relation with the medial olfactory nucleus and proceeds near the ventromedial surfaces of the hemispheres. Here it becomes approximated laterally with the lateral forebrain bundle. Bundles of fibres appear on the medial side of the precommissural septal or parolfactory area, proceed ventrally and joins the medial forebrain bundle. Dorsomedial hemisphere wall gives rise to corticoseptal bundles which proceed into the precommissural septal area and join the medial forebrain bundle.

*Birds :*

Tractus septocorticalis and tractus corticoseptalis interconnect septal areas and other basal regions of the medial telencephalic wall with the hippocampus. Bundle of fibres proceeding from septal areas to the pre-optic and hypothalamic regions is known as medial forebrain bundle. Tractus septomesencephalicus or corticoseptomesencephalicus connects the medial hemisphere wall with the ventrolateral wall, diencephalic regions and the mesencephalic regions.



*Mammals :*

Valverde (1965) states that the medial forebrain bundle is formed by a long system of fibres and short-axoned cell system. The common pathway throughout the regio praeoptica and hypothalamus of the following five components forms the first system :

- (a) Fibers from the cortex pyriformis.
- (b) Axons from the cluster of cells of the tuberculum olfactorium.
- (c) Fibers of giant pyramidal cells of the frontal cortex.
- (d) The hypothalamic component of the stria terminalis.
- (e) Fibers from cells of the septal nuclei.

Cells extending from the regio praeoptica and lateral hypothalamus to mesencephalic levels form the system of short links of the medial forebrain bundle. It includes the bed nucleus of the medial forebrain bundle. It is difficult to state whether axons of cells of the nucleus of the diagonal band of Broca enter into the medial forebrain bundle.

#### HOMOLOGIES OF THE CORTICAL AREAS IN REPTILES, BIRDS AND MAMMALS (Kuhlenbeck, 1929)

Nucleus basalis (paleostriatum), nucleus basalis accumbens, nucleus epibasalis and nucleus centralis have been compared by him with the basal ganglia of mammals. Cortex medialis of reptiles and birds has been homologized with the hippocampal formation of mammals. The medial part of the cortex dorsalis of reptiles has been compared with the dorso-medial cortex of birds. Mammalian neocortex has been compared to the parahippocampal cortex and cortex dorsalis pars intermedialis of reptiles and mammalian regio insularis has been compared to the cortex dorsolateralis of reptiles.

*Bird archistriatum :*

It is situated in the posterior third of the lateral hemisphere wall under the neostriatum and caudal to the paleostriatum. It can be divided into dorsal and ventral portions. The nucleus taeniae appears near the anterior end of the archistriatum. It can be considered as a part of the archistriatal complex.

The hyperstriatum accessorium is situated within the "Wulst or Sagittal Wulst". "The paleostriatum primitivum is the homologue of the globus pallidus of higher-forms. The homologies of the paleostriatum augmentatum are less clear. It may include within it the primitive portions of both the caudate nucleus and the putamen (Huber and Crosby)."



*Reptilian septal area, amygdaloid complex (archistriatum), hippocampus, piriform lobe cortex, fornix longus and the fornix, pallial commissures, stria medullaris and stria terminalis :*

The nucleus olfactorius anterior is present and the tuberculum olfactorium has been noted in some reptiles with differentiation into three layers.

The striatal areas are more advanced than those noted in amphibians.

The anterior end of the hypopallial ridge is somatic and represents the head of the caudate and the putamen. The posterior end is olfactory and along with some basal centers represents amygdaloid complex of mammals and may also represent claustrum.

The hippocampal area is developed well and the connections are through the fornix and fornix longus systems. The piriform lobe cortex and the general cortex have also been noted. The parahippocampal area of Dart can be differentiated from parapiiform cortex of Dart.

#### *Septal area :*

The septum is homologous with the parolfactory areas and this is not the same as septum pellucidum of higher vertebrates. Towards the posterior part the septum can be divided into a medial and a lateral portion. There are nuclei lateralis septi, nuclei medialis septi and the nucleus septohippocampalis.

Between the hypothalamic areas and the tuberculum olfactorium there is the preoptic region.

#### *The amygdaloid complex :*

The hypopallial ridge of *Sphenodon* is the same as the dorsal ventricular ridge of turtles. This ridge can be divided into cephalic and caudal portions. The cephalic portion is divided from an infolding of the general pallium. The posterior portion (amygdaloid ridge) is derived from an ingrowth of the piriform lobe cortex.

#### *Hippocampus :*

In the lizard there is a medial and a dorsal portion in the hippocampus. "The medial or dorsomedial portion, as described in the lizard by Ariens Kappers and de Lange appears to be homologous with the pars dorsomedialis hippocampi of the alligator and the hippocampus proper of the turtle." Projection fibres of fornix type starts from the dorsomedial portion of the hippocampal region. There is a dorsal portion which forms association cortex.

#### *Piriform lobe cortex :*

The anterior end of the piriform lobe cortex is noted at the cephalic end of the hemisphere, lateral to the pars dorsalis hippocampi. This cortex extends backwards towards the posterior pole of the hemisphere.



*The fornix longus and the fornix :*

There is anlagen of the fimbria and fornix system and pallial commissures by fibres arising from the dorsomedial and dorsal hemisphere. There are also fibres which can be compared to the septomesencephalic tract of birds. The corticohypothalamic tract is thought to represent the fornix column of higher forms. They partially decussate in the pallial commissure and enter the diencephalon. Some fibres enter the stria medullaris (tractus cortico habenularis medialis pars inferior). Other remaining fibres proceed caudally and there is partial decussation in the retroinfundibular or supramammillary commissure. At the caudal end of the hypothalamus they end in the mammillary body (de Lange). This bundle may also end in other hypothalamic areas (lateral, ventral and periventricular nuclei).

*Pallial commissures :*

The anterior pallial (hippocampal) commissure is situated dorsal to the anterior commissure. The posterior pallial commissure is found in sphenodon, snakes and lizards.

*Stria medullaris*—It has got the following components :

- (1) Tractus corticohabenularis medialis (tractus septohabenularis).
- (2) Tractus corticohabenularis medialis pars inferior .
- (3) Tractus cortico (and amygdalo) habenularis lateralis anterior.
- (4) Tractus corticohabenularis lateralis posterior (tractus amygdalo-habenularis lateralis posterior).
- (5) Tractus olfactohabenularis medialis pars preoptica and pars hypothalamica.
- (6) Tractus olfactohabenularis lateralis.
- (7) Tractus olfactohabenularis posterior.

*Stria terminalis :*

In the lizard and the alligator this can be divided into :

- (a) Pars commissuralis, and
- (b) Pars preoptica et hypothalamica.

*Cerebral cortex of reptiles* was described by S. R. Cajal in "Histologie du systeme nerveux de l'homme & des vertèbres. Tome II, pages 836-842." There are different regions of the reptilian cerebral cortex—supero-internal region called mediodorsal cortex ; one laterodorsal region ; one inferior or basilar region ; and one internal region or *fissuraire*. In the frontal section of the supero-internal region one can see : (1) superficial plexiform zone ; (2) pyramidal cells ; (3) deep plexiform zone ; (4) one layer of white substance and (5) one ventricular ependyme. In figure 534 all these five layers have been depicted in the cerebral frontal section



of *Chamaeleo vulgaris* (Golgi method). In figure 535 septal region of the same animal has been shown. There is fundamental ganglion or *corps strie* below the ventricle. Pyramidal cells of the internal region of the cortex (fascia dentata of certain authors) are seen. Basal bundle has been noted and its fibres are seen to ramify into the fundamental ganglion. There are also cells which envelop the basal bundle. Figure 536 shows the frontal section of the brain of the lizard (*Lacerta stirpium*) (Golgi method). Anterior commissural fibres, sagittal bundle or *mescencephalique* d'Edinger, direct descending fibres to the basal bundle, inferior fibres of the anterior commissure, outgoing commissural fibres of the spherical nucleus and other types of fibres are noted.

In the fourth layer or *substance blanche* P. Ramón described four different paths :

- (1) Homolateral association path formed by the external branches of bifurcation of the axiscylinders issuing from the pyramidal cells. These branches probably terminate in the external cortical region or laterodorsal cortex.
- (2) Longitudinal association path or sagittal bundle. This is situated in the median plane and found below the interhemispheric fissure. It terminates in the occipital region of the brain and can be compared to mammalian cingulum.
- (3) Crossed association path or *corps calleux*.
- (4) Direct and crossed projection path. "On voit la figure 536 les fibres directes, FD, et les fibres croisées, FC qui constituent cette voie et que P. Ramón, puis Edinger ont décrites. On est peut-être en droit d'identifier cette voie avec le *fornix longus de Forel* des vertébrés supérieurs, c'est-à-dire avec la partie du cingulum qui est formée de fibres de projection. On pourrait aussi la considérer comme une ébranche des piliers antérieurs du trigone, si l'on admet avec Edinger que l'écorce supéro-interne des reptiles correspond à la corne d'Amon et à la fascia dentate des mammifères."

The anatomy of the rhinencephalon is a very short summary of "Anatomie du Rhinencephale" by Professors H. Gastaut and H. J. Lammers (1961) and published by Masson et cie, Editeurs for which the present author is indebted to them greatly and also for permitting to use some figures in this book.

In the fish (Fig. R6) the archipallium is represented by primordium hippocampi and the paleopallium by primordium piriforme. Neopallium is not developed.

In the dipnoans and batracians (Fig. R6B) the primordium neopallii is situated on the dorsal surface of the hemisphere in between the archipallium and the palaeopallium.

In the reptiles (Fig. R6C) the primordium neopallii is situated on the dorsal part of the hemisphere as a thin band of cortex in between the archipallium and the palaeopallium. The palaeopallium is represented



by piriform lobe. The archipallium is distinguished topographically by hippocampal area occupying the internal face of the hemisphere and is separated from the paraterminal body (ept.) by one Sulcus called parolfactory posterior Sulcus (s.p.p.). One parahippocampal area (parahipp.) is the lateral prolongation of the previous area and is in contact with the neopallium. Cytologically the hippocampal area is already differentiated into one lateral part of predominantly big pyramidal cells which is equivalent to cornu d' Ammon and one medial part of predominantly small granular cells equivalent to dentate gyrus.

The conditions in mammals (Monotremes and Marsupials) are shown in Fig. R6D and Figs. R8, R9, also show the differentiation of telencephalic zones.

#### *Isocortex and allocortex (Figs. R20-R23) :*

Comparative names for isocortex is "homogenetic cortex" and that for allocortex is "heterogenetic cortex". During ontogenetic development the allocortex does not go through six-layer stage. Palaeopallium and archipallium are examples of allocortices. Brodmann included olfactory bulb, parolfactory area of Broca, anterior perforated space, hippocampus and the different associated areas including the entorhinal cortex under his "heterogenetic cortex." These areas are known as rhinencephalon. Heterogenetic cortex is subdivided into cortex primitivus, cortex rudimentarius, and cortex striatus.

Isocortex or homogenetic cortex is characterized ontogenetically by a six-layered pattern of lamination. Crosby *et al.* (1962) described further subdivisions.

Rhinencephalon is divided into the following by Gastaut and Lammers (1961).

1. Pars basalis rhinencephali (Lobus olfactorius).
  - A. Lobus olfactorius Anterior :
    - (1) Bulbus olfactorius.
    - (2) Gyrus olfactorius communis.
    - (3) Gyrus olfactorius medialis and lateralis + tractus olfactorii.
    - (4) Area olfactoria.
      - (a) Tuberculum olfactorium.
      - (b) Substantia perforata anterior.
      - (c) Gyrus diagonalis.
    - (5) Area septalis.
  - B. Lobus olfactorius posterior (Lobus piriformis).
    - (1) Gyrus parahippocampi.
      - (a) Pars entorhinalis and paraesubicularis = gyrus parahippocampi proper.
      - (b) Pars uncinata (peri—amygdaloidea) = gyrus uncinatus.
    - (2) Amygdala.



- II. Pars limbica rhinencephali (Lobus limbicus Fig. R38).
- A. Limbus hippocampi (corticalis) (Fig. R22).
  - (1) Pars paraecommissuralis.
  - (2) Pars supracommissuralis (indusium griseum).
  - (3) Pars retrocommissuralis=formatio hippocampi (Figs. R23 and R24).
    - (a) Subiculum.
    - (b) Hippocampus proper=cornu Ammonis.
    - (c) Gyrus dentatus.
    - (d) Gyrus fasciolaris ; gyrus intralimbicus.
- Limbus medullaris.
  - (1) Fimbria
  - (2) Fornix
- C. Limbus chorioideus.

The vascularization of the rhinencephalon is complex and originates from majority of the arteries of the brain.

Anterior cerebral artery irrigates the olfactory bulb and its peduncle, medial olfactory circumvolution, antero-internal part of the anterior perforated space, pre and supracommissural hippocampal vestiges, medial part of orbital lobe, parolfactory area and anterior cingulate region. The septal area is irrigated by arteries from anterior communicating artery.

The middle cerebral artery supplies the major part of the parahrinal region and, in particular, the lateral part of the orbital lobe and anterior perforated space, lateral olfactory circumvolution, insula and retro-insular area, temporal lobe tip and anterior part of the superior and middle temporal convolution.

The anterior choroidal artery supplies the diagonal gyrus and through one special arterial pedicle the uncus and the amygdala are supplied (sometimes also the ventral part of the cornu d' Ammon).

The posterior cerebral artery supplies the inferior temporal circumvolution, the fusiform gyrus and the parahippocampal gyrus with the exception of uncus. The artery finally irrigates chiefly the hippocampal formation, particularly the Ammon's horn and the gyrus dentatus. Particular attention has been cast on the vascularization of the hippocampus because of elective vulnerability of the pyramidal cells of the Ammon's horn and particularly of the sector of Sommer in certain pathological conditions (epilepsy, different circulatory troubles, carbon monoxide intoxication, neurosyphilis etc.). These have been studied in man, monkey, cat, dog, rabbit and opossum and Nilges (1944)



studied the arterial supply of the hippocampus on a comparative basis. Ushimura (1928) had identical observation on man (Fig. R25).

Connections of rhinencephalon have been depicted in Figs. R26, R27, R28 and R29.

### *Amygdala :*

The following nuclear groups and subdivisions have been noted in the amygdala.

Lateral nucleus is composed of five territories : gracilis, limitans, magnocellularis, mediocellularis and parvocellularis.

Basal nucleus is subdivided into three parts.

- (1) Ventromedian nucleus.
- (2) Basolateral or dorsolateral nucleus has four territories : magnocellular, mediocellular, parvocellular and microcellular.
- (3) Accessory basal nucleus.

Central nucleus comprises two territories : median or magnocellular and lateral or microcellular.

Medial nucleus.

Cortical nucleus.

Intercalated nucleus.

Nucleus of the lateral olfactory fascicle.

Nucleus of the stria terminalis.

One anterior amygdaloid area.

### CONNECTIONS OF THE AMYGDALA

#### *Afferent paths to the amygdala :*

A. The *olfactory* fibres are anatomically well defined. They originate in the olfactory bulb and end in the cortical and medial amygdaloid nucleus and the nucleus of the lateral olfactory stria of the same side, also to the central and basomedial nucleus and bed nucleus of the stria terminalis of both sides. Sumtotally therefore, corticomедial and basomedial nucleus of the amygdala receive olfactory afferents of the same or both sides, whereas basolateral and lateral nucleus and anterior amygdaloid areas are totally devoid of the same.

#### *(B) Nonolfactory sensory connections of amygdala :*

These are visual, auditory, gustatory, somesthetic, visceresthetic. These have been demonstrated electrophysiologically.

C. Nonsensory afferents arise from the subjacent pyriform cortex (gyrus parahippocampi) and the adjoining temporal and perirhinal regions.





Parahippocampal (Piriform) cortex gives rise to *parahippocampo-amygdalar path* comparable to *parahippocampo-ammoniacal path*. Lammers and Lohman (1957) proved that fibres from periamygdaloid cortex pass to the lateral nucleus and the basal nucleus of the amygdala.

Afferent amygdaloid paths from temporal cortex have been described by different authors. Whitlock and Nauta (1956) observed in *Macacus* projections from inferior temporal gyrus to the basolateral amygdaloid nucleus. Segundo, Naquet and Arana (1955) thought that the projections proceed from the superior temporal gyrus to the same nucleus. Lammers and Lohman (1957) demonstrated the existence of fibres from temporal pole to the lateral nucleus (chiefly in the dorsal part) and the central nucleus of the amygdala.

Amygdalar afferents also come from perirhinal cortex (Posterior orbital cortex, anterior insular and temporopolar) and these have been demonstrated by physiological neuronography (Pribram, Lennox and Dunsmore, 1950 ; Kaada, 1951 ; Gastaut, Naquet and Roger, 1952 ; and Gloor, 1955).

*Efferent connections (after Gastaut and Lammers, 1961 and Gloor, 1960).*

Subcortical connections comprise a dorsal (*the stria terminalis*) and the *ventral amygdalo subcortical pathways*. The stria terminalis is divisible into four components in relation to anterior commissure. They are commissural, supracommissural, infracommissural and postcommissural ones. Main endings of the stria terminalis are in the septal and preoptic area, the anterior hypothalamus and the ventromedial hypothalamic nucleus. Connections to other hypothalamic areas and nuclei posteriorly up to the premammillary region are possible.

*Ventral amygdalobulbar pathways :*

Some end in the tuberculum olfactorium and the septum coursing with the diagonal band of Broca. Some fibres enter the median forebrain bundle. Some are associated with the longitudinal association bundle and with the anterior commissure. The ventral fibres end in the bed nucleus of the stria terminalis, the septal, preoptic and anterior hypothalamic regions. Fibre connections with entopeduncular nucleus, the subthalamus or the brain-stem tegmentum may also occur. Connections may also exist to the putamen and claustrum and to some thalamic nuclei (pulvinar, dorsomedial, lateralis posterior and lateralis dorsalis nuclei).

Connections from the basolateral amygdaloid complex to the piriform cortex have been established. Amygdalohippocampal connections are said to exist. Amygdaloid fibres also proceed to the cingulum, the tip of the temporal lobe and the insula.

Intimate relationship of the amygdala with highly integrative subcortical structures has been noted by analysis of the neuronal organiza-



tion of the amygdaloid projection systems (Gloor's Fig. 5, 1961—from Gloor, 1955. Fig. R 31). Fig. R 30 illustrates electrophysiologically demonstrated direct and indirect connections of the amygdala and their functional significance (Fig. R 32).

#### *Commissural connections :*

Amygdalae of two sides are interconnected by fibres of the anterior commissure. These fibres proceed to the commissure directly. The fibres forming the commissural part of the stria terminalis proceed at first with this bundle and then cross the anterior commissure. Previously it was thought that the amygdaloid fibres coursing directly into the anterior commissure originate from the basolateral amygdala but recently it has been demonstrated that they originate from the corticomedial complex instead.

By electrical stimulation of the amygdala, subcortical propagation has been noted by Magnus and Naquet (1961), (Fig. R 39). Vegetative responses have been depicted by them in Figs. R 40, R 41, R 42, R 43 and by Monnier and Gangloff (1961) in Fig. R 44.

#### *Lesion experiments (Gloor, 1960) :*

Changes in adrenocortical response have been separately dealt with.

After bilateral amygdaloid lesions there are transient disturbances in the sleep-wakefulness cycle and there is prolonged sleep-like states and disturbances of a narcoleptic character.

After bilateral anterior temporal lobectomy including amygdala in monkeys, Kluver and Bucy (1937) described a syndrome consisting of (a) visual agnosia, (b) hypermetamorphosis (a strong urge to attend and react to every visual stimulus), (c) oral compulsive behaviour, (d) changes in emotional behaviour with loss of fear and of aggressiveness, (e) hypersexuality, (f) changes in dietary habits and the animals accepted meat as food, and (g) increased and peculiar spontaneous motor activity.

Bilateral amygdaloid lesions made the animals more placid and they did not show reactions of fear, rage or aggression. Spiegel *et al.* (1940) and Bard and Mountcastle (1948) found that after bilateral amygdalotomy in cats the placid behaviour was changed into savage one. Aggressiveness may be due to increased sexual behaviour. Schreiner and Kling (1953, 1954) observed that subsequent castration abolished hypersexuality and docility was restored. Green, Clemente and De Groot (1957) offered another explanation for the discrepancy in behaviour. They think that inadvertent injury to the blood supply of anterior hippocampus may lead to savage behaviour, whereas similar injury to blood vessels of basal ganglia may give rise to apathy which resembles placidity. Schreiner and Kling (Fig. R 37) observed hypersexuality after bilateral amygdaloid lesions in male cats. Copulation was attempted with animals of other species or with other males. No aggressiveness is shown towards a dog and instead approach is made as a potential sexual mate. Green *et al.* (1957) studied experimental hypersexuality in cats and observed



the effects of small electrolytic and surgical lesions in the amygdala, hippocampus, piriform cortex and stria terminalis. Destruction of amygdala alone could not produce hypersexuality. Small lesions in the piriform cortex situated just medial to the rhinal fissure and beneath the basolateral amygdaloid nucleus produced hypersexual changes and there was no damage to the amygdala. Hypersexuality develops after the lapse of several weeks. Increased copulatory and abnormal sexual behaviour (Fig. R 36). Such as homosexual activities, masturbation, and attempts at copulation with animals of other species or outside territory have been noted. Hypersexuality is prevented to develop by preoperative castration. In amygdalectomized animals increased sex hormone production is not the cause of hypersexuality. Subcortical activities in the female rabbits during sexual activities have been demonstrated by Green (1961). Electrical activities in the brain of rabbits have been obtained during the course of coitus (Figs. R 33, R 34, R 35). There appears to show a great deal of activity of the hippocampus during preliminary activity and activity of the anterior hypothalamus in a more late stage, and in course of copulation.

### *The Hippocampus :*

Connections of the hippocampus have been discussed by Green (1960). Fig. R 30 represents diagrammatically the areas which have afferent and efferent connections with hippocampal formation. *Vertical hatchings* on the left side of each section of the brain denote areas which when stimulated give rise to responses in the hippocampus which are always bilateral. *Crosshatched areas* on the right side of each section are those areas which are bilaterally excited by stimulation of the hippocampus. On hippocampal stimulation *stippled areas* are ipsilaterally excited. These are based on experiments on the cat by Green and Adey (1956).

Green and Arduini (1954) described various afferent responses in the hippocampus in acute rabbit preparations under curare and local anaesthesia.

MacLean (1961) deposited carbachol crystal in the hippocampus of cats (Horsley-Clarke' coordinates in the frontal plane A 1.5 and A 4.5).

The subsequent modifications can be divided into three stages. The stage of development is noted in the upper three tracings of the figure, the stage of culmination in the middle and the stage of retrocession in the basal three tracings.

In Fig. R 45, Monnier and Gangloff (1961) while discussing the integrative functions of rhinencephalon showed distribution of the responses to electrical stimulation (3/sec.) of the hippocampus (RH). Specially to note is the generalised appearance of the points at the level of the cortex, in the thalamus (TH) and in the mesencephalic reticular formation (FR).



*Development and structure of the pituitary of reptiles :*

Wingstrand (1951, 1963, 1966) has studied the comparative anatomy and evolution of the hypophysis in details and the following observations have been adopted from his works. The anterior pituitary is developed from the hollow pouch of Rathke and its top comes in approximation with the developing neural lobe. The pouch gets broader near the top and the base and thus forms an aboral and an oral lobe. The two lobes are separated by a constriction. An epithelial stalk connects with the oral ectoderm. Two lateral lobes grow towards the eminentia from the oral lobe near the constriction. The oral lobe has an anterior process and thus the adenohypophyseal anlage takes up the shape of a 'U'. That part of Rathke's pouch which is in contact with the neural lobe gives rise to pars intermedia. The remaining portion of aboral lobe and oral lobe except the lobi laterales constitute pars distalis. The lateral lobes get themselves attached to the eminentia mediana and become the pars tuberalis. In *Lacerta* the lobi laterales reach the brain and are represented by two cell masses which are embedded in the surface of the brain and loose contact with the main gland (Baumgartner, 1916). The tuberalis is completely reduced in some other species. In the snakes there is no development of pars tuberalis. Hypophysiportal vessels in Lacertilians and the snakes proceed from the median eminence to the pars distalis through a connective tissue string called the pars terminalis and there is no tuberalis tissue in it. The snake pituitary has a compact neural lobe and it is strongly asymmetrical.

Szentagothai and Szekely (1958) observed in lizards a very clear indication of transmission of anterior pituitary substances to the median eminence which might be an important function of pars tuberalis. The pars tuberalis of lizards could be easily traced. The two lateral protrusions of Rathke's pouch are closely approximated to the infundibular process of the third ventricle and are incorporated into the median eminence of the lizards. The cell groups look like hypothalamic nuclei and easily mistaken for the retrochiasmatic part of the supraoptic nuclei. These cell groups secrete Bodian's protargol-staining granulae. Similar findings have been noted in *Calotes versicolor*.

The chromophobes of the anterior pituitary have argyrophilic granulae. Some relationship exists between the granulae (Bodian-positive) and ACTH secretion. In the cat there is quick increase of argyrophilic granulae in chromophobe cells on the second postadrenalectomy day. In lizards the pars tuberalis cells within the median eminence have plenty of intracellular Bodian-positive granulae. There is discharge of these granules from the pars tuberalis cells into the median eminence. Bodian-positive granules in the cellular processes are discharged into the median eminence by a process akin to clasmatodendrosis. The authors could note migration of such cells laden with argyrophilic granulae into the median eminence. Such pictures are frequently noted after adrenalectomy or transection of the hypophyseal stalk and also found in *Calotes versicolor*.



Wingstrand (1951) said, "A comparison between the structural plan of the adenohypophysis in birds and reptiles shows, that the caudal lobe of the adult avian pituitary corresponds to the intermedia and the adjacent part of the pars distalis in reptiles because the material in both cases is supplied by the aboral lobe. The cephalic lobe in the avian pituitary corresponds to the rostral part of the reptilian pars distalis, and this part is well developed in reptiles. The lobi laterales give rise to a pars tuberalis in birds, crocodiles, chelonians and Rhynchocephalis, but do not form a tuberalis in the snakes, in which they are indistinct already in small embryos, and they are partly or completely reduced also in lizards."

The thickness of pars intermedia is variable in Lacertilians. Pandalai (1966) observed large, well developed and active pars intermedia in the garden lizard, *Calotes versicolor*. The intermedia cells vary in staining properties. They may be slightly basophilic (cyanophilic) or chromophobic in *Lacerta* and strongly cyanophilic in *Anguis*. They are deeply acidophilic in *Agama* and *Varanus* and Geckos they are strongly chromophilic (deep violet colour after Azan staining) (Wingstrand, 1966).

The pars distalis is subdivided into a cephalic lobe and a caudal lobe. Wingstrand (1966) observed coarse-granulated acidophils in the caudal lobe of *Xanthusia* and *Anguis*, whereas most deep-staining acidophils are noted in the cephalic lobe of *Anolis* and *Varanus*. Resorcin-fuchsin stained basophils are strikingly restricted to the cephalic lobe of *Anguis*. Saint Girons (1963) studied the comparative histology of the reptilian adenohypophysis. Six cellular categories were identified including the cells of the intermediate lobe. In the pars distalis there are LH gonadotropic cells, FSH gonadotropes, thyrotropes, alpha and X (epsilon. ?) cells. Localization of different cellular categories of the pars distalis is very constant. Haematoxylin always stains LH gonadotropic cells and these cells react most frequently with aldehyde fuchsin. In numerous cases X cells react slightly with PAS. Grignon (1963) studied the adenohypophysis of the land turtle (*T. mauritanica*) and Viper (*V. aspis*). Beta and gamma cells have gonadotropic significance. FSH and ICSH are elaborated in the beta and gamma cells respectively. Castration in the male and female sexes confirms the gonadotropic nature of the beta cells. Delta cells have a thyrotropic significance. These cells are active with the thyroid gland activity and vacuolation is noted in them after thyroidectomy or treatment by antithyroid drugs. Corticotropic hormones are perhaps elaborated by the alpha cells and in the turtle gonadotropic function is ascribed to the erythrosinophilic cells. The X cells in *Vipera aspis* should secrete somatotrophic hormone.

In *Calotes versicolor* pituitary, at the rostral region there are cells belonging to the acidophilic series which can be stained with Orange G and lead haematoxylin. They are morphologically similar to the lactotrope. Their corticotropic nature is established by responses to metyrapone treatment where hypertrophy and hyperplasia have been noted. They are distributed along the secondary capillary nets.

The post-optic part of the hypothalamic floor gives rise to the neurohypophysis by an evagination of the posteroventral wall called the saccus



infundibuli (Wingstrand, 1966). The base of the saccus infundibuli corresponds to the infundibular stem. The median eminence is located between the chiasma and the saccus infundibuli; but an undifferentiated area remains in between the median eminence and the chiasma called the "pars oralis tuberis" in the majority of reptiles. Majority of the neurosecretory fibres from the supraoptico-hypophysial tract end in the neural lobe having a close contact with the adenohypophysis and this has been called "distale adeno-neuro-hypophysare Kontaktfläche" by Spatz *et al.* (1948) and Spatz (1953). The end of the saccus infundibuli becomes forked or T-shaped and thus producing a pair of primary branches (Wingstrand, 1951, 1959). The growth of the neural lobe takes place by thickening of the walls of the primary branches or nervous material diffusely migrates into the surrounding mesenchyma and thus a thick neural lobe is formed in the snakes and mammals where pituicytes are found. In sphenodon, many reptiles and birds, the neural lobe is thin walled. There is ependymal layer and no formation of free glia cells. The wall consists of an ependymal layer, intermediate fibre layer (coarse fibres of preoptic-hypophysial tract) and a superficial palisade layer having endings of neurosecretory axons and crossed by processes of ependymal cells.

#### *Median eminence :*

The median eminence of some lizards is thin and there is very little proliferation. It has got an inner ependymal layer, intermediate fibre layer and outer palisade layer covered by capillaries. There are few or no pituicytes. Some reptiles have thick median eminence and plenty of pituicytes and capillaries form loops into the wall. Snakes have a typical median eminence but there is no pars tuberalis. Remnants of the pars tuberalis are located in the sulcus tubero-infundibularis on the margin or outside the median eminence in lizards. Wingstrand (1966), therefore, said, "The contact with the pars tuberalis is thus a doubtful criterion for defining the eminentia." Vascular contact (Portal) between the eminentia and the adenohypophysis is present in the lizard and this forms a "proximale neuro-adenohypophysare Kontaktfläche" of Spatz *et al.* (1948) and Spatz (1953, 1958). According to Spatz there are two zones of contact between the hypothalamus and the adenohypophysis. Pars tuberalis (infundibularis) of the adenohypophysis is closely applied to the ventral surface of the infundibulum. The other contact zone is between the infundibular process and the adenohypophysial pars intermedia. Detailed description has been given by Diepen (1962).

#### HYPOTHALAMIC NUCLEAR MASSES IN REPTILES AND FIBRE CONNECTIONS (KAPPERS *et al.*, 1967)

The preoptic area is continuous behind with the hypothalamic area without any break.

The following nuclear masses are noted: the nucleus periventricularis hypothalami, the nucleus hypothalamicus anterior, the nucleus hypothalamicus lateralis and the nucleus hypothalamicus ventralis.



At the anterior end of the hypothalamus deeply stained neurons of the periventricular hypothalamic nucleus fuse with the interstitial cells of the olfactory projection tract. This nucleus is present throughout the whole extent of the hypothalamus.

The lateral hypothalamic nucleus is situated in front of the habenular commissure, ventral to the forebrain bundles and lateral to the periventricular hypothalamic nucleus. Posteriorly it extends slightly behind the habenular commissure. It consists of medium sized cells. Anteriorly this group is continuous as the anterior hypothalamic nucleus. These two groups receive forebrain fibres.

The ventral hypothalamic nucleus is related to the ventral hypothalamic commissural system. At the posterior end of the hypothalamus there is a deeply staining compact nucleus which de Lange called the corpus mammillare because he could trace the fornix bundle to it and it was found to be connected with the anterior thalamic nucleus and it was regarded as the tractus mamillothalamicus or Vicq d' Azyr fasciculus. However, Kappers *et al.* (1967) could not detect such a connection in the Alligator. Diepen (1962) showed in his figure No. 82 a and b on page 130 the horizontal section through the tuber cinereum and the caudal anlage of the corpus mammillare of *Lacerta viridis*. Bigger nerve cells within the medullated fibres are spoken of as the elements of nucleus mammillaris lateralis of mammals. Possibility of smaller nerve cells lying near the ventricle in group as anlage of medial mammillary nucleus is there. Similar findings are there in the mammillary nucleus of *Calotes versicolor* (Figs. R 12, R 13, R 14).

A fornix system from the projection cells of the hippocampal region to the hypothalamic areas has been described by many (Figs. R 11, R 15 show the same in *Calotes versicolor*). On the medial wall of the hemisphere the basal olfactory centers are interconnected with the hypothalamic centers by hypothalamic component of the medial forebrain bundle (*C. versicolor*). "The amygdaloid complex, the nucleus of the lateral olfactory tract and the piriform lobe complex are interrelated with preoptic, hypothalamic, and perhaps midbrain areas by way of the stria terminalis and olfactory projection paths" (also in *C. versicolor*). Striohypothalamic component of lateral forebrain fibres proceed to the lateral and anterior hypothalamic nuclei. Connection of the hypothalamic areas with the tectum by periventricular systems is probable. There are also ascending hypothalamic fibres from lower centres.

#### NEUROSECRETORY SYSTEM IN REPTILES

These have been described by Hild (1950, 1951), Diepen (1952, 1955), Bargmann (1954, 1955), Ghiara (1954-1957), E. and B. Scharrer (1954), Roy (1958) and others.

The hypothalamo-hypophyseal pathway starts from the supraoptic and paraventricular nuclei in reptilia. The neurosecretory cells extend one of their processes towards the ventricular cavity. Gabe (1966) stated that the neurosecretory perikaryons in Reptilia are not so prominent as



noted in Anamniota and the secretory events in them are not so spectacular as noted in Teleostei and in anurous Batrachia. Bargmann *et al.* (1950) noted chrome haematoxylin-positive secretion granules in the perinuclear regions of the supraoptic and paraventricular nuclei of Ophidia. Nissl bodies are located at the peripheral regions of the cytoplasm and Dräger (1949) noted vacuoles in the same area, though the significance of them are not yet clear.

The same groups of neurosecretory cells show more or less identical features in Lacertilia.

In chelonians (*Testudo graeca*, and *Emys europaea*) the paraventricular nucleus is very large and long dendrites run from the cells towards the ventricular wall.

The hypothalamo-hypophyseal tract is formed by the convergence of the fibres from the neurosecretory cells and they proceed towards the floor of the third ventricle. The ventral wall of the infundibulum corresponds to the median eminence of homoiothermal vertebrates. There are ependymal, fibrous and glandular layers.

Microscopic anatomy of the neurohypophysis in Reptilia shows wide variations, (Green, 1951; Saint Girons, 1961). The infundibular recess does not extend into the large neurohypophysis in Ophidia. This lobe is divided by connective tissue septa into small lobules. Mostly in other reptiles the infundibular recess enters into the neurohypophysis to a greater or lesser extent. Digitations proceed from the dorsoventrally flattened sac which contact with the adenohypophyseal pars intermedia. The neurohypophysis contains neurosecretory products, terminations of the hypothalamo-hypophyseal tract, and pituicytes.

#### ULTRASTRUCTURE OF THE NEUROSECRETORY SYSTEM IN REPTILIA

Murakami (1961) studied the neurosecretory cells in the hypothalamus of the lacertilian, *Geco japonicus*. The preoptic nucleus of *Geco* consisted of big neurosecretory cells which showed considerable development of ergastoplasm and contained osmiophil secretory granules. The clefts between the ns cells and the neuroglial cells or other neurones measured between 100 and 150A. The ergastoplasm is irregular; sacs of different sizes occupy the position of the lamellar structure. The sacs contain structures of about 4000A in diameter. These structures are composed of very osmiophil granules of diameters ranging between 900 and 1200A. These are thought to be neurosecretory granules. The granules are separated from the ergastoplasmic membranes by a clear space. The Golgi apparatus is juxtannuclearly situated. Cisternae, vacuoles and vesicles have been recognized which contain osmiophil granular inclusions. The chondriomes are of usual appearance.

Chondriosomes, elementary granules and inclusions as noted in the Golgi complex of the perikaryons, have been noted in the hypothalamo-hypophyseal tract. Endoplasmic reticulum and ribosomes have, however, not been noted in this location by Murakami (1961).





Histochemical, electron microscopic and pharmacologic studies on the median eminence of the fish, frog, turtle, bird and laboratory rat were conducted by Kobayashi (1965). AF-positive neurosecretory material has been noted in the fish (*Oryzias latipes*), neurohypophysis and the external layer of the median eminence of the frog (*Rana catesbeiana*), turtle (*Clemmys japonica*) and birds (pigeon, grass parakeet). There are neurohaemal regions in the fish neurohypophysis and the median eminence of the frog where axon endings contain large electron-dense neurosecretory granules (about 1400A) and synaptic vesicle-like structures. There are also a few axon endings here which contain small electron-dense granules (about 800A) and synaptic vesicle-like structures. In the median eminence of the turtle and birds there are axon endings which contain few large electron-dense granules but plenty of small electron-dense granules. In the mouse and the rat few aldehyde fuchsin positive neurosecretory axons are seen in the external layer of the median eminence. But there are many axon bulbs containing small electron-dense granules and synaptic vesicle-like structures. Axon endings having large electron-dense granules are rarely found in this layer of the median eminence. Axon endings containing mostly synaptic vesicle-like structures were also thought to be present in the median eminence and the pars nervosa of the animals.

Small electron-dense granules (about 800A) in the external layer of the median eminence of the mouse and rat may be carriers of catecholamines (Kobayashi, 1965). Granules in the supraoptico-hypophyseal tract and in the pars nervosa are much larger than the small electron-dense granules in the median eminence. Large electron-dense granules are neurosecretory granules which carry neurohypophyseal hormones. Small electron-dense granules of the neurohypophysis of the fish and of the external layer of the median eminence of the turtle and birds may be carriers of catecholamines. Some axon terminals in the median eminence may contain both catecholamine granules and synaptic vesicle-like structures containing acetylcholine. Arrangement of the synaptic vesicle-like structures may vary from *diffuse type* to *cluster type* in the axon bulbs, clusters aggregate against the inner surface of the membrane of the axon bulb which is situated against the pericapillary connective tissue space. "These *active points* may be involved in the permeability changes of the membranes of the axon bulbs through the release of ACh, thus facilitating, directly or indirectly, the passage of neurohypophyseal hormones or catecholamines through the membrane." Bulbs containing few empty large or small granules have smaller number of synaptic vesicle-like structures. Whereas bulbs containing many large or small electron-dense granules are associated with many synaptic vesicle-like structures.

Kobayashi (1965) thought that the adeno-hypophyseal hormone-releasing factors are present "in granules of the same size as either the large or small electron-dense granules".

Birgmann, Knoop and Thiel (1957) studied the structure of the neurohypophysis in Reptilia (ophidian *Natrix natrix*). There are unmyelinated fibres with cytoplasm containing elementary granules of diameter ranging from 1500 to 3000A, chondriosomes and neurofilaments.



The cytoplasm of the pituicytes has much larger inclusions which have a tendency to disintegrate and thereby produce lamellar formations.

### EXPERIMENTS ON THE HYPOTHALAMUS OF REPTILES

Callard and Willard (1969) studied the effects of intrahypothalamic betamethazone implants on adrenal function in male *Sceloporus cyanogenys*. The findings suggest that the hypothalamus contains steroid-sensitive receptor cells which control adrenal size through the anterior pituitary gland.

Plasma corticosterone levels in the male iguanid lizard *Sceloporus cyanogenys* were noted by Daugherty and Callard (1972) under various physiological conditions. Control baseline levels of the steroid in plasma were significantly decreased by hypophysectomy, adrenalectomy, hypothalamic lesions and cyanoketone. Levels were increased after treatment with mammalian ACTH in both intact and hypophysectomized lizards.

Experimental results in *Calotes versicolor* (personal observations)  
(Figs. R 17, R 18, R 19)

Experimental conditions	Plasma corticosterone level
1. Mammalian ACTH (2 IU/ animal) 31°C	Rise at 1/2 hr.
2. ACTH injection in hypophysectomized animals (8 days).	Rise at 1 hr. from a low level.
3. ACTH in dexamethasone-blocked animals.	Rise at 1/2 hr.
4. Metyrapone injection total 60 mg. in 7 days.	Hypertrophy of adrenal glands.
5. Hypophysectomy .. ..	Fall
6. Adrenalectomy .. ..	Fall
7. Betamethazone implants in the hypothalamus (ventromedial nucleus and infundibular nucleus) and median eminence.	Fall
8. Stress (fracture of Rt. femur*) ..	Rise
9. (a) Stimulation of the hippocampus ..	Fall
(b) Stimulation of the hippocampus + stress (*).	Rise (insignificant)
10. (a) Lesion of the hippocampus ..	Rise
(b) Lesion of the hippocampus + stress (*).	Further rise
11. Stimulation of ventromedial nucleus, infundibular nucleus and median eminence.	Rise
12. Lesion of the abovementioned areas ..	Fall
13. Stimulation of septal area ..	Fall





Experimental conditions	Plasma corticosterone level
14. Lesion of septal area .. ..	Rise
15. Stimulation of archistriatum .. ..	Rise
16. Lesion of archistriatum .. ..	Fall
17. Pituitary grafts in the mediobasal hypothalamus (ventromedial nucleus, infundibular nucleus) and median eminence.	Grafts well maintained and rise in plasma corticosterone level.
18. Do. + stress (*) .. ..	Further rise

Roy (1958) stressed the importance of the hypophyseoportal circulation in the control of the anterior pituitary by the median eminence and hypothalamus with special reference to the adrenocortical function in the garden lizard, *Calotes versicolor* and changes in them after different forms of stress (fracture, scald and ether anaesthesia).

*Calotes versicolor* has got well-marked median eminence and the primary capillary net of the portal vessels is partly within it and partly on the surface of it. Through the pars terminalis the portal vessels enter into the pars distalis where they break up into secondary capillary net. On the surface of the median eminence there are basophilic cells similar to those noted in pars tuberalis. Wingstrand (1951) stated that his investigations made him believe that the pars tuberalis really has a function. In lizards and birds some cells in the pars tuberalis are packed with argyrophilic granules. He said, "the pars tuberalis is functional whenever present, but that its function cannot be essential for the maintenance of the amniote organism".

Roy further stated that there is well-developed neural lobe and pars intermedia in the garden lizards. *The direction of flow of blood in the hypophyseoportal vessels is from the median eminence towards the pars distalis.* Nerve fibres containing neurosecretory substance are found to end around the primary capillary net in the portion of the infundibulum which corresponds to the median eminence in higher vertebrates. "These findings help in the postulation of the idea that neurosecretory substance comes into the pars distalis through the hypophyseoportal vessels and a part contained in the substance stimulates the pars distalis to produce ACTH or gonadotrophin or other hormones."

Roy (1958) noted the neurosecretory substance (CAHP stain of Gomori) in the following situation in *Calotes versicolor* :

- (a) in the neurosecretory cells of the hypothalamus as granules and along the axons of the cells.
- (b) in the extracellular spaces.
- (c) towards the adjoining ventricle.
- (d) upward extension from the hypothalamic level.
- (e) neural lobe of the pituitary.
- (f) in the richly vascularized median eminence region.



Stress led to depletion of neurosecretory substance. The restorative phase occurred after some time. The vacuolar change in the neurosecretory cells is most commonly met with after-stress.

The interrenal cells are small and contain lipid. The gland is very vascular. Stress leads to congestion of the organ and loss of sudanophilic substance from the interrenal cells. Vacuolar change in these cells has also been noted after stress.

COMPARISON OF THE NEUROHYPOPHYSIS AND ITS INNERVATION IN  
BIRDS, REPTILES, MAMMALS AND LOWER VERTEBRATES  
(WINGSTRAND, 1951)

The neural lobe is formed by proliferation of the top of the saccus infundibuli in mammals, birds and reptiles and it is homologous throughout. Green (1947, 1951) defined the median eminence as a part of the neurohypophysis which is coextensive with the primary capillary net of the hypophyseoportal system and having a typical histological appearance.

Nowakowski (1951) defined the "infundibulum" of the cat as a part of the diencephalic floor which coextends with the pars tuberalis. The "infundibulum" is delimited by a sulcus infundibularis from the surroundings and has a characteristic histological structure differing from the nearby parts of the brain. Dense capillary net was on the surface of it. Wingstrand (1951) states that this area corresponds well with the median eminence as described by Green and Wingstrand.

Wingstrand (1951) states that Nowakowski's infundibulum (median eminence) may be coextensive with the pars tuberalis in the cat and some other mammals, but it is not so in all mammals. The pars tuberalis extends high up by the sides of the tuber cinereum in birds but it is frequently absent from the central and most typical parts of the median eminence. Pars tuberalis is not present in snakes, but they have a distinct and typical eminentia with characteristic histological structure and vascularization. Distinct sulcus infundibularis is absent in many reptiles and birds.

Wingstrand (1951) defines the median eminence as that part of the diencephalic floor which is coextensive with the primary plexus of the hypophyseoportal system of blood vessels and characterized by a superficial glandular layer.

Well-defined ventral wall of the third ventricle extends from the chiasma (supra-optic decussations) rostrally to the saccus infundibuli caudally. Median eminence of the adult cat covers most of this surface except a small part just behind the chiasma. Neuro-hypophyseal structure is not found in this part and it is called *pars oralis tuberis* by Nowakowski (1951). In birds the whole ventral wall from the chiasma to the saccus is differentiated as a median eminence except in the duck and goose, in which case a small *pars oralis tuberis* is noted just behind the chiasma. In reptiles (lizards and snakes) a very small part of the post-optic diencephalic floor forms the median eminence and therefore the *pars oralis tuberis* extends more caudally. In the amphibians the *pars*



oralis tuberis is very large as the median eminence is a small portion near the pituitary.

Wingstrand (1951) stated, "Nowakowski's infundibulum is the same as my eminentia mediana, his radix infundibuli is my transitional zones, his pars infundibularis is my pars tuberalis, and his Zwischenstück is my infundibular stem".

Nowakowski's external or peripheral zone of the median eminence is the same as the glandular zone of Wingstrand and his central zone is the same as Wingstrand's fibre layer and ependymal layer.

Nowakowski's peripheral zone is innervated by delicate fibres from the nearby parts of the tuber nuclei, the nuclei tuberis infundibularis. These nuclei correspond to the ventral parts or more of the nuclei tuberis in birds.

"The peripheral zone of the cat median eminence has little or nothing to do with the neurosecretory neurons of the tr. supraoptico-hypophyseus, which mainly terminates in the *Hinterlappen* (neural lobe)." The *Radix infundibuli* is a transitional zone between the *infundibulum* and the tuber cinereum. The surface of this area has a neurohypophyseal structure and the nucleus tuberis is situated in deeper layers.

In the neurohypophysis of the teleosts there are non-neurosecretory and neurosecretory fibres and glia cells. Most of the neurosecretory fibres pass to the meta-adenohypophysis and a few may pass into the pro and meso-adenohypophysis. Fibres to meso-adenohypophysis are distinct. Diepen (1962) thinks that the anterior ramifications of the neurohypophysis consisting mainly of non-neurosecretory fibres should be considered as modified median eminence (infundibulum) as these form a type of proximal adenoneurohypophyseal contact.

Wingstrand (1966) states that in most cases the pituitary gland is supplied by vessels entering around the stem of the neurohypophysis and ramifying along its branches to all parts of the adenohypophysis. Direct arterial supply into the pro and meso-adenohypophysis has also been noted. Vessels may originate from a ring artery around the stem. Wingstrand (1966) said, "On the way to the pituitary, some capillaries or capillary systems may be in close contact with eminentia-like structures around the stem base or along the ramifications of the neurohypophysis".

Regarding the homologies of the teleost neurohypophysis he says that the saccus infundibuli of embryos forming the neural lobe of tetrapods is represented mainly by the saccus vasculosus of adult teleosts. In the caudal ramifications of the teleost neurohypophysis there is neurosecretory substance, suggesting thereby that this part is the functional equivalent of the neural lobe. "Thus, when comparing the *basic morphological pattern* in the neurohypophyseal regions of teleosts and amniotes, the neural lobe of amniotes may be homologized with the saccus vasculosus of fish. On the other hand, when the *functional system* of the neural lobe with its neurosecretory nerve endings is considered the neural lobe of amniotes may be compared with the neurosecretory part of the neurohypophysis of fish, although the latter is situated far in front of the embryonic saccus infundibuli in some species."





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## CHAPTER 15

### LEGENDS TO FIGURES

- FIG. R1 Le plan fondamental du telencephale et son evolution. a, telencephale primaire ; b, debut de l'ovagination ; c, telencephale inverse. 1, archipallium ; 2, paleopallium ; 3, striatum ; 4, region septale ; d, le telencephale everse (d'apres A. Kappers).
- FIG. R2 Schema montrant l'evolution de l'archicortex. A, Amph bien ; B, Rat ; C, Homme. Hachures obliques : archipallium (hippocampe). Hachures quadrillage : region septale (d'apres Krieg).
- FIG. R3 Coup longitudinale a travers l'encephale du Lizard et interessant en particulier le cervelet.
- FIG. R4 Cerveau de Cobra (*Hamadryas hannah*). A, on remarque, immediatement en arriere des volumineux corps bijumeaux (tectum opticum), les corpora postica, venant affleurer en surface ; B, meme cerveau, apres enlevement du cortex de l'hemisphere droit. On aperçoit le striatum, divise en neostriatum (rostral) et archistriatum (caudal).
- FIG. R7 Schema montrant l'apparition du neopallium et l'invagination de l'archipallium. A, Reptile ; B, Mammifere Eutherien. 1, archipallium ; 2, paleopallium ; 3, neopallium ; 4, neostriatum ; 5, archistriatum ; 6, paleostriatum ; 7, commissure anterieure ; 8, commissure superieure ou commissure de l'hippocampe ; 9, fibres neopalliales dans la capsule interne ; F. Rh., fissure rhinale (d'apres Portmann).
- FIG. R8. La differenciation des quatre zones telencephaliques :
- (a) *Batracien* : *Necturus* (Kappers, fig. 564).
  - (b) *Reptile* : *Alligator* (Kappers, fig. 578). Coupes frontales en avant du diencéphale.
  - (c) *Marsupial* : *Hypsigimnus* (Kappers, fig. 267). Enroulement de l'hippocampe, absence de corps calleux. Le pallidum appartient au trigone, la commissure anterieure, ventrale, est interbulbaire,—hippocampique et—piriforme. Les fissures hippocampique et rhinale limitent le neo-cortex.
  - (d) *Rat* : d'apres Gurdjian. Mammifere a corps calleux ; coupe frontale passant par les noyaux habénulaires. La separation du diencéphale est majorée a gauche. L'hippocampe est nettement situe sous le corps calleux, sans vestiges supra-calleux. La fleche en pointille indique le trajet du fornix vers les corps mamillaires.
- FIG. R 10. Sagittal section of the brain of *C. versicolor*.
- FIG. R 11. Transverse section through forebrain of *Cajal's versicolor* (Kluver-Barrera stain). It shows the optic chiasma below, the medial and lateral forebrain bundles on both sides and the crossing fibres of the commissural system.
- FIG. R 12. Horizontal section of the brain of *C. versicolor*. It shows the ventromedial nucleus, infundibular recess and nucleus infundibularis.
- FIG. R 13. Horizontal section through the tuber cinereum of *C. versicolor* (Kluver-Barrera stain). Rostrally it shows the chiasma and supraoptic commissure and caudally a rectangular space is marked in which medial and lateral anlage of mamillary nucleus is noted. The enlarged view of this portion is seen in Fig. R 14.
- FIG. R 14. Caudally the big nerve cells form the lateral mammillary nucleus. The small nerve cells rostrally and more medially above the commissural fibres and below the ventricular recess are the anlage of the medial mammillary nucleus.
- FIG. R 15. Transverse section of the forebrain of *C. versicolor*.
- FIG. R 23. 1. Archicortex ; 2. paleocortex ; 3. periarhicortex (pars ventralis) ; 4. periarhicortex (pars dorsalis) ; 5. peripaleocortex ; 6. juxtaarchicortex (pars ventralis) ; 7. juxtaarchicortex (pars dorsalis) ; 8. juxtapaleocortex.



Fig. R 30. Diagrammatic representation of areas which are in afferent or efferent connections with the hippocampal formation. *Vertical hatching areas* on the left side of each brain section, when stimulated, evoke responses in the hippocampus which are always bilateral. *Crosshatched areas* on the right side of each section are excited bilaterally by hippocampal stimulation. Stimulation of the hippocampus excites the *stippled areas* ipsilaterally. (From Green and Adey, E. E. G. Clin., Neurophysiol., 1956, 8, 257.)

Fig. R 31. Schematic representation of the neuronal organization of the amygdaloid projection system (by electrophysiological studies). The dotted area represents the subcortical integrative areas which regulate *global* mechanisms and the limbic structures projecting into it.

Ac : N. accumbens. Am, b-l : Subdivision baso-laterale du complexe amygdalien. Am, m : Subdivision corticomediale du complexe amygdalien. An : N. antérieurs du thalamus. Cd : N. caude. CI : Collicule inférieur. Cm : Centre median. CS : Collicule supérieur. GL : Corps genouille externe. GM : Corps genouille interne. Ha : Hypothalamus antérieur. Hip : Hippocampe. Hy : Hypothalamus postérieur. IL : Noyaux thalamiques intralaminaires. L.P : N. lateral postérieur. MD : N. dorsomedian. Mes : Mesencephale. Mm : Corps mammillaire. NHvm : N. hypothalamique ventromedian. Pul : Pulvinar. R : N. reticulaire du thalamus. Ret : Substance reticulée du tronc cérébral. RPo : Région preoptique. Spt : Septum. VA : N. ventral antérieur. VM : N. ventro-median. (Tire de Gloor, E. E. G. Clin. Neurophysiol., 1955, 7, 233).

Fig. R 32. Schema montrant les relations entre le complexe amygdalien et son système de projection sous-cortical et les relations fonctionnelles qui en découlent (Tiere de Gloor, E. E. G. Clin. Neurophysiol., 1955, 240).

Figs. R 33, R 34 and R 35. Activité sous-corticale de la femelle du lapin durant l'activité sexuelle.

RF RO —derivation fronto-occipitale droite.  
LF LO —derivation fronto-occipitale gauche.  
R and L HIP —hippocampe droit et gauche.  
A and PHYP —hypothalamus antérieur et postérieur.  
Buck —male.  
Doe —femelle.

Fig. R 33. Traces de contrôle. La flèche indique la stimulation par un "click". Noter la réaction d'éveil et le rythme theta dans l'hippocampe et l'hypothalamus. Deuxième trace : même réaction d'éveil quand un male est placé dans la cage.

Fig. R 34. Premier trace, la femelle pourchasse le male. Noter le male. Noter les rythmes theta dans l'hippocampe et l'hypothalamus postérieur. Deuxième trace, le male chasse la femelle. Pas d'activité dans l'hypothalamus antérieur (la chasse commence approximativement au moment où le trace se modifie).

Fig. R 35. Premier trace, giration précédant l'accouplement. De nouveau, noter le trace de l'hypothalamus antérieur.

Deuxième trace, le coit commence peu après le début du trace. Noter l'activité rapide d'amplitude élevée dans l'hypothalamus antérieur au commencement du coit.

(Figs. R 33, R 34 and R 35 are from Green, 1961 : "Physiologie et Pathologie du Rhinencephale" Masson. Paris).

Fig. R 36. Activité sexuelle anormale suivant des lésions rhinencephaliques.

- 1 —Tentative de copulation avec un objet inanimé. Lésion du cortex piriforme.
- 2 and 3 —Le même animal avec un chat anesthésié et un chaton.
- 4 —Masturbation. Lésion du cortex piriforme.
- 5 —Trois animaux normaux ; au sommet un animal porteur de lésion dans le cortex piriforme.



- 6 —Avec un lapin. Lésion dans le cortex piriforme et l'amygdale.  
 7 to 9 —Comportement pervers consécutif à une lésion du cortex piriforme avec castration.  
 7 —le mâle ignore l'oestrus de la femelle.  
 8 —Il préfère un jouet à l'oestrus de la femelle.  
 9 —Il préfère l'oestrus de la femelle au jouet, 24 heures après l'injection intra-musculaire de 1 mg de propionate de testostérone. (From Green, 1961. "Physiologie et Pathologie du Rhinencéphale". Masson. Paris).

Fig. R 37. Bizarre sexual reactions of male cats after bilateral ablation of the fronto-temporal region (Schreiner and Kling, 1953) (From Mac Lean, 1961. "Physiologie et Pathologie du Rhinencéphale". Masson. Paris).

Fig. R 38. From Mac Lean, 1961. "Physiologie et Pathologie du Rhinencéphale." Masson. Paris.

Fig. R38 (a) Ce dessin d'une vue de la face interne et inférieure du cerveau de la loutre est la première figure de l'article de BROCA de 1878. La région non encadrée correspond à ce qu'il a défini comme le grand lobe limbique. Comme il le dit : (Le nom... que j'ai adopté indique les rapports constants de cette circonvolution avec le limbe de l'hémisphère ; il n'implique aucune théorie... il est applicable à tous les cerveaux des mammifères, à ceux qui ont un vrai corps calleux comme à ceux dont le corps calleux est nul ou rudimentaire...).

Fig. R38 (b) Dans les dessins ci-dessus ou les faces externe et interne des cerveaux du lapin (A), du chat (B) et du singe (C) sont dessinées approximativement à l'échelle le lobe limbique est représenté en noir. La figure illustre le fait que le lobe limbique, comme BROCA l'a indiqué, forme un dénominateur commun du cerveau de tous de tous les mammifères. On remarquera comme le lobe entoure le tronc cérébral, position qui a suggéré à BROCA l'emploi du terme (limbique) (d'après Mac Lean. In Wittk...).

Figs. 39 to 43—From Magnus and Naquet, 1961. "Physiologie et Pathologie du Rhinencéphale." Masson. Paris.

Fig. R 40. Schémas anatomiques représentant l'amygdale ou sont notés les points positifs ayant provoqué du reniflement, signalés par les différents auteurs.

Figs. R 44 to R 45. —From Monnier and Gangloff, 1961. "Physiologie et Pathologie du Rhinencéphale." Masson. Paris.

Fig. R 45. Répartition des ripostes à la stimulation électrique (3/sec) de l'hippocampe (RH). On notera surtout l'apparition généralisée de pointes au niveau du cortex, dans le thalamus (TH) et dans la formation réticulaire mésencéphalique (FR).



# Chapter 15

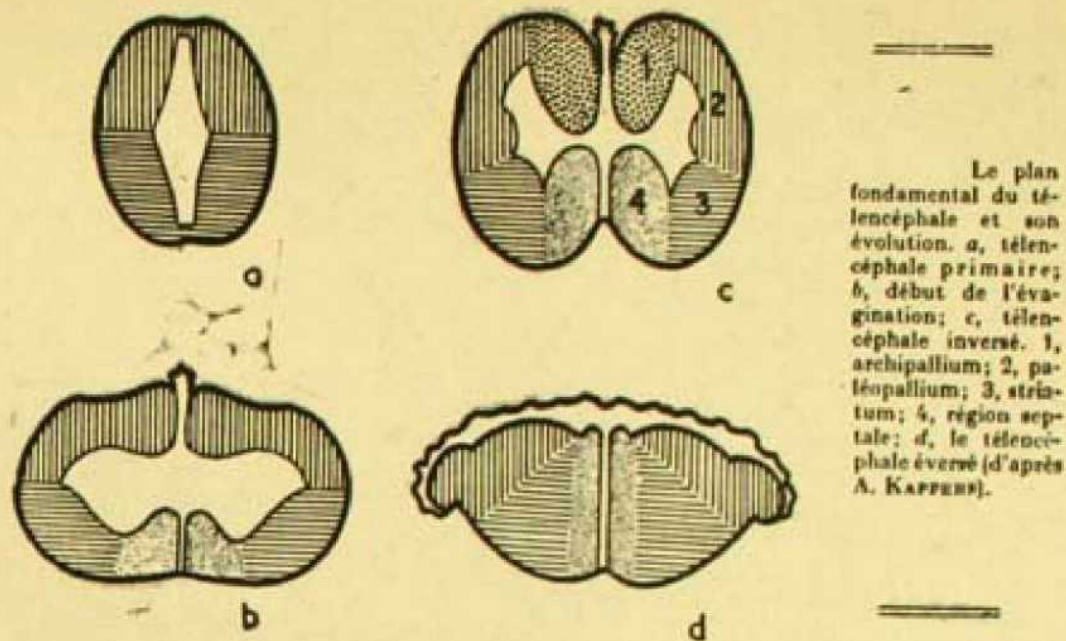


FIG. R 1

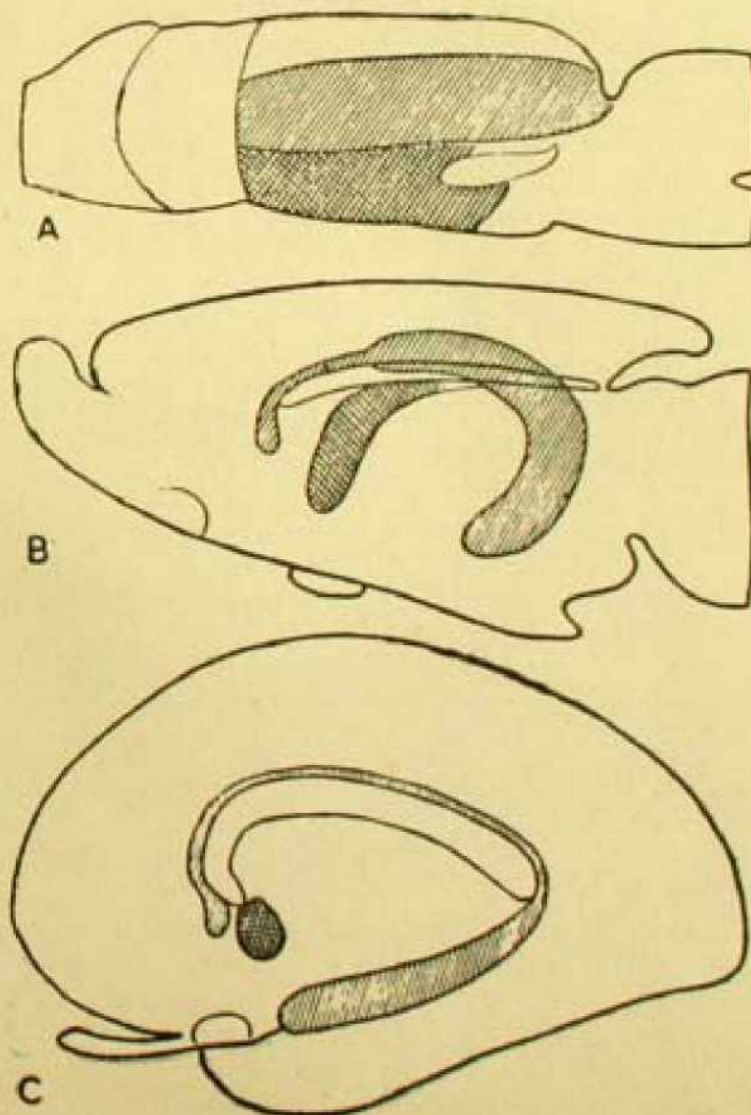


Schéma montrant l'évolution de l'archicortex. A, Amphibien; B, Rat; C, Homme. Explication dans le texte. Hachures obliques : archipallium (hippocampe). Hachures en quadrillage : région septale (d'après KARRER).

FIG. R 2



Fig. 19



Fig. 19

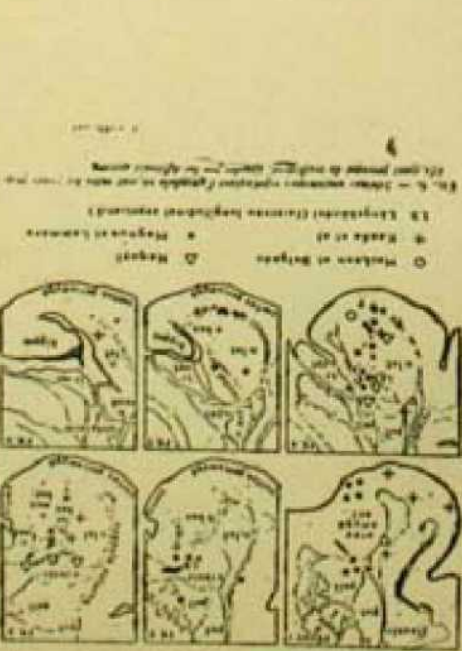


Fig. 19

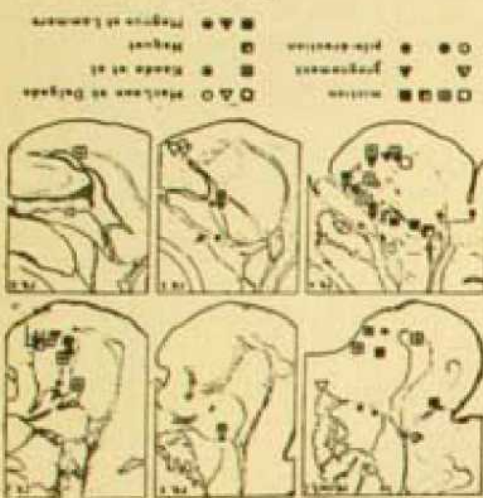


Fig. 19

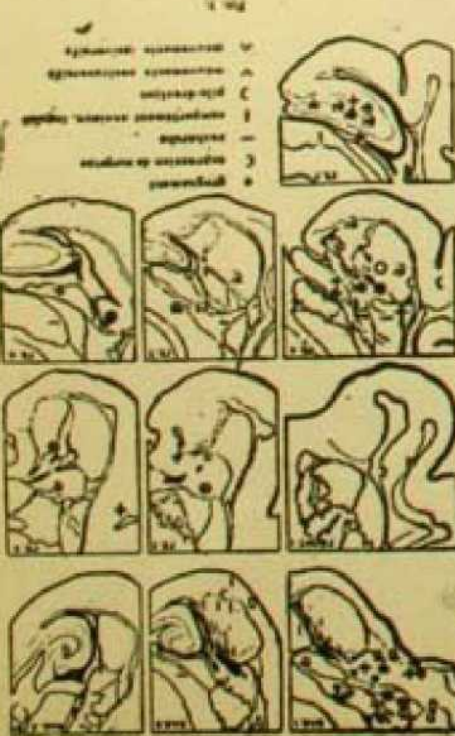




Fig. 11. 39

— Schéma destiné à montrer les aires enroulées par les post-décharges obtenues après stimulation électrique de l'amygdale (Figure empruntée à ARANA et coll., 1951).

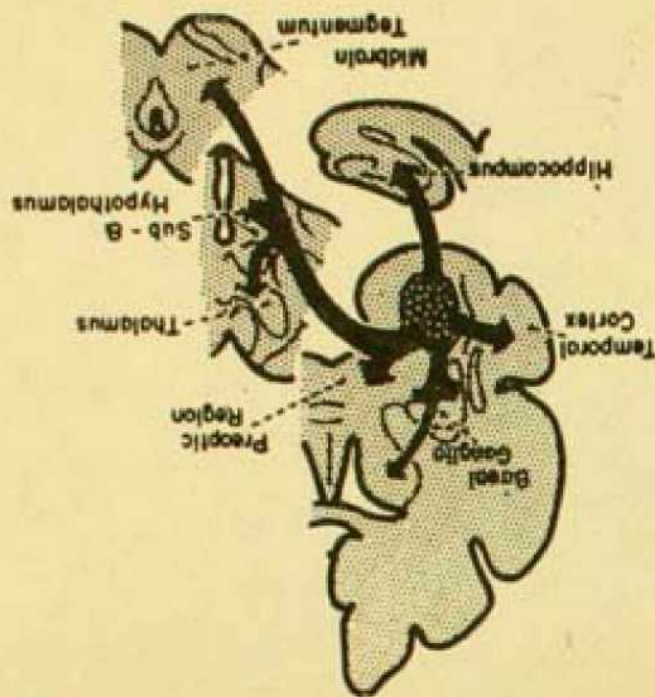


Fig. 12. 38

Figure 12. 38. Schéma de l'amygdale et de ses connexions. Les zones de l'amygdale sont désignées par des lettres. Les zones de l'amygdale sont désignées par des lettres. Les zones de l'amygdale sont désignées par des lettres.



Figure 12. 38. Schéma de l'amygdale et de ses connexions. Les zones de l'amygdale sont désignées par des lettres. Les zones de l'amygdale sont désignées par des lettres. Les zones de l'amygdale sont désignées par des lettres.

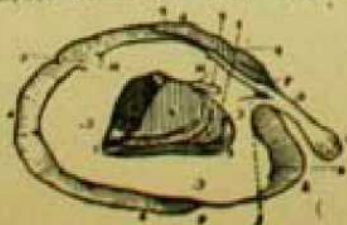




FIG. 11 37

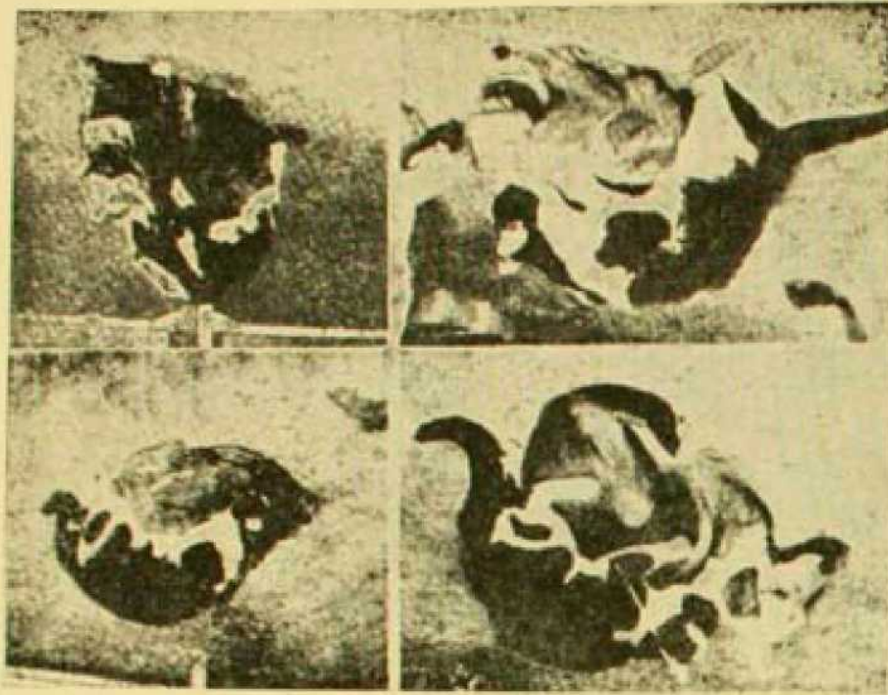
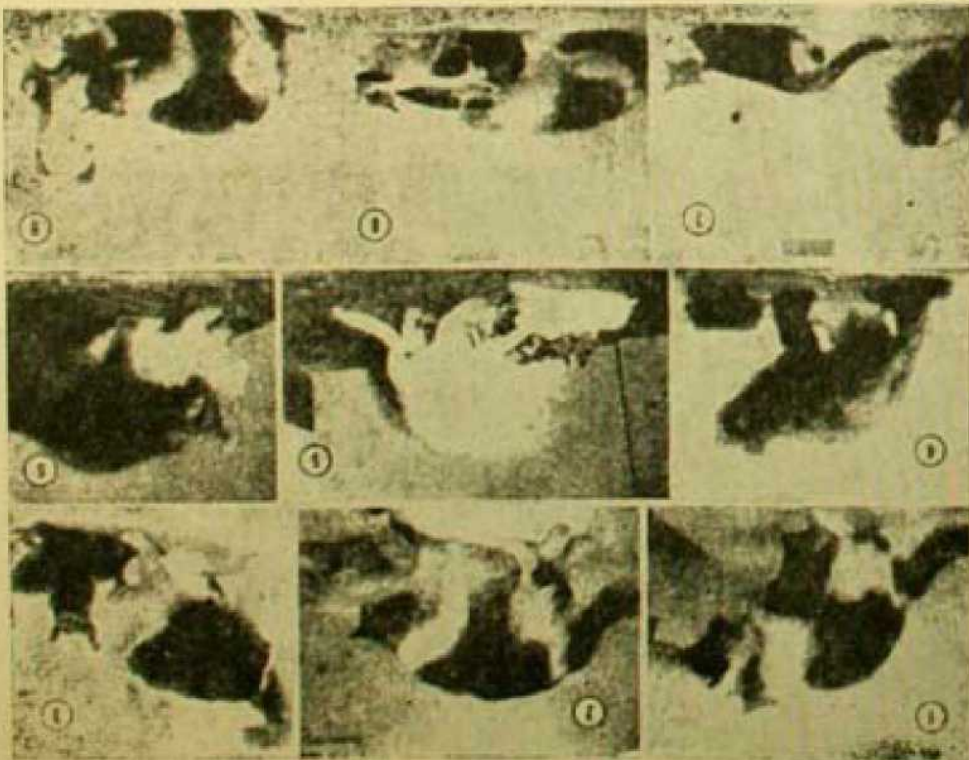


FIG. 11 38





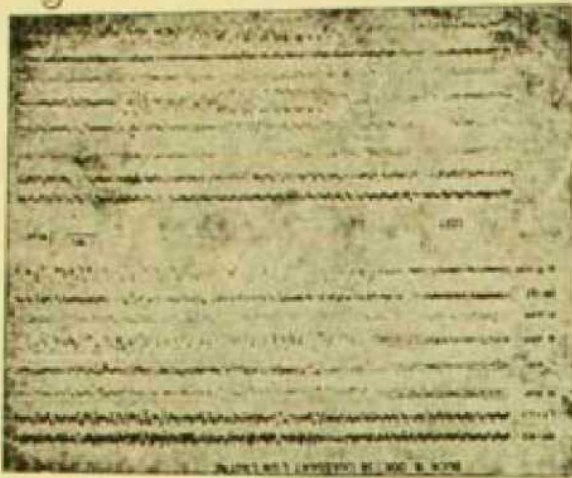


FIG. R 35

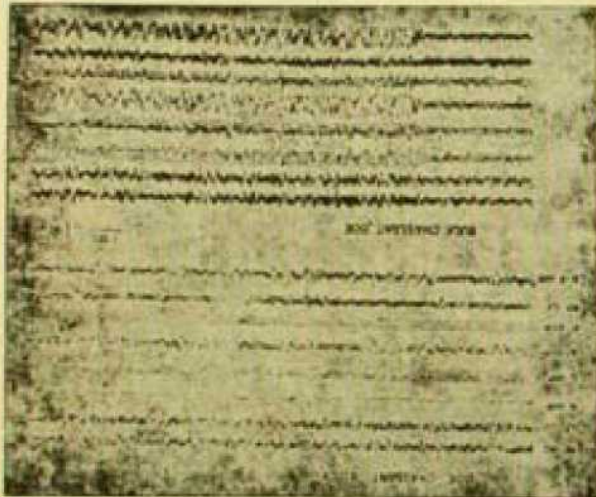


FIG. R 34

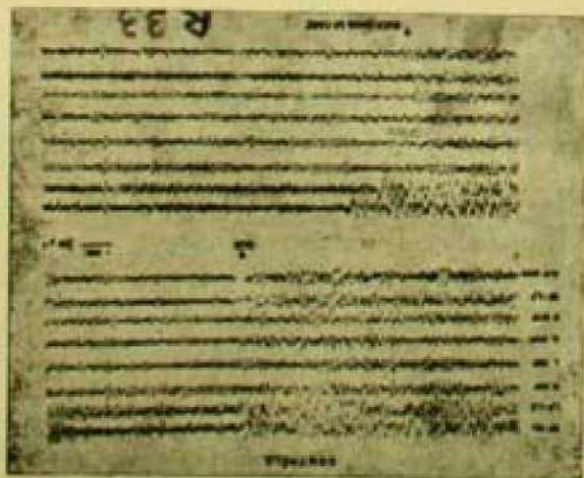


FIG. R 33



Fig. 6. — *Feldspar montmorillonite* with *kaolinite* and *illite* in *clay* (see text).

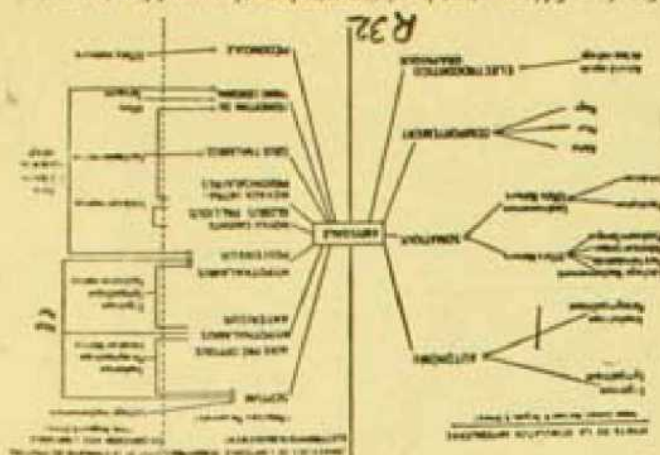
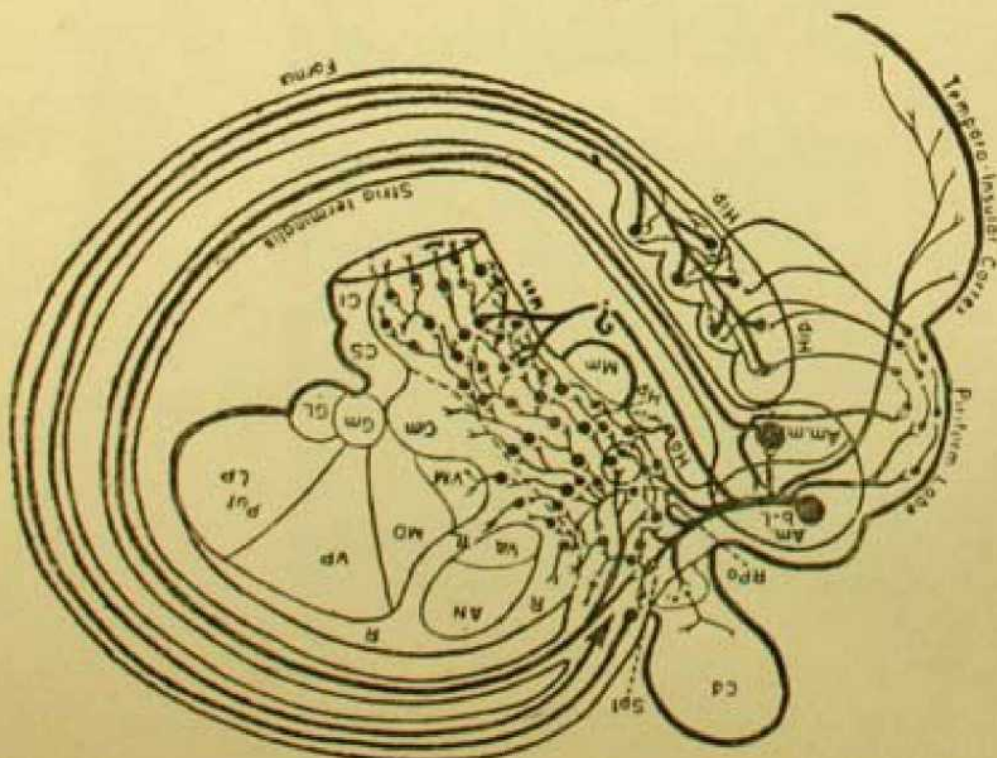


Fig. 34 16 341

Représentation schématisque de l'organisation  
du système de projection amygdalique (basé sur des expériences sur le chat).



Représentation diagrammatique des aires  
en connexion afférente et efférente avec la formation hippocampale.

Fig. R 31

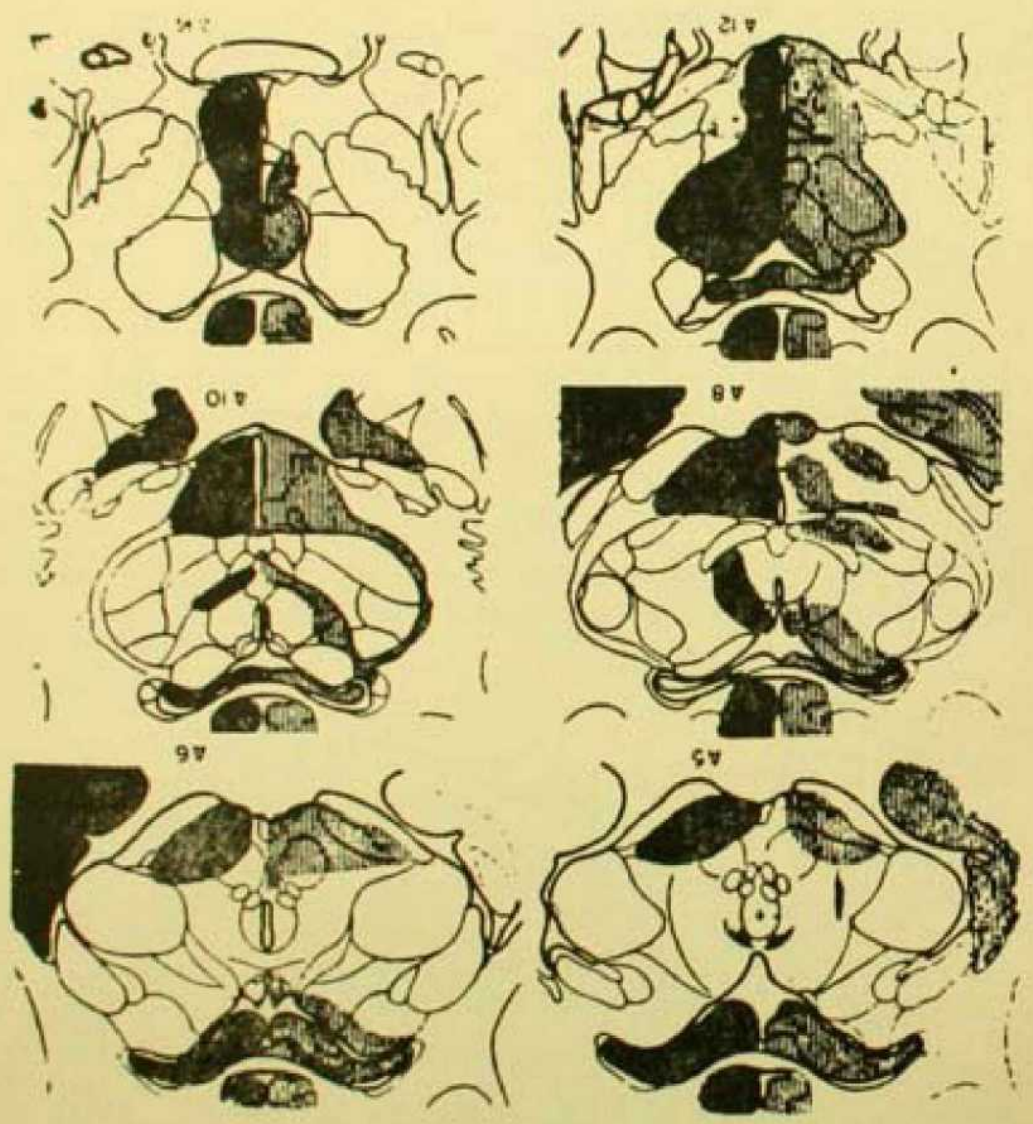
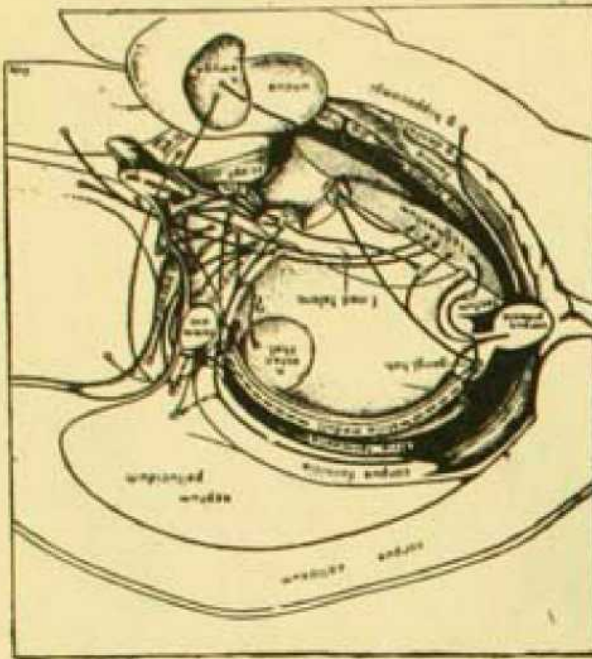
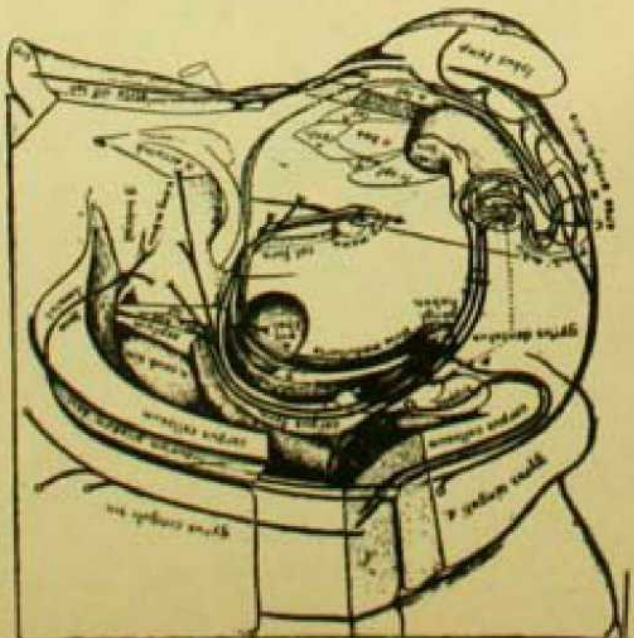




Fig. 11 59



15 H 97



10. 28 28





Fig. H 26

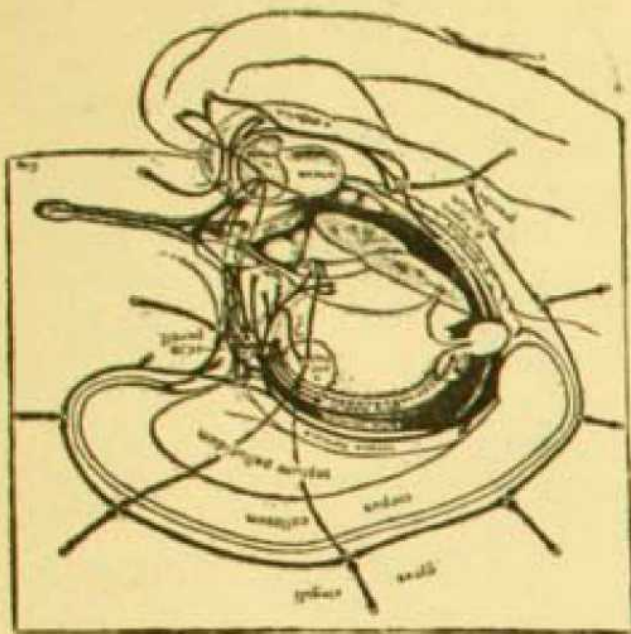


Fig. H 25

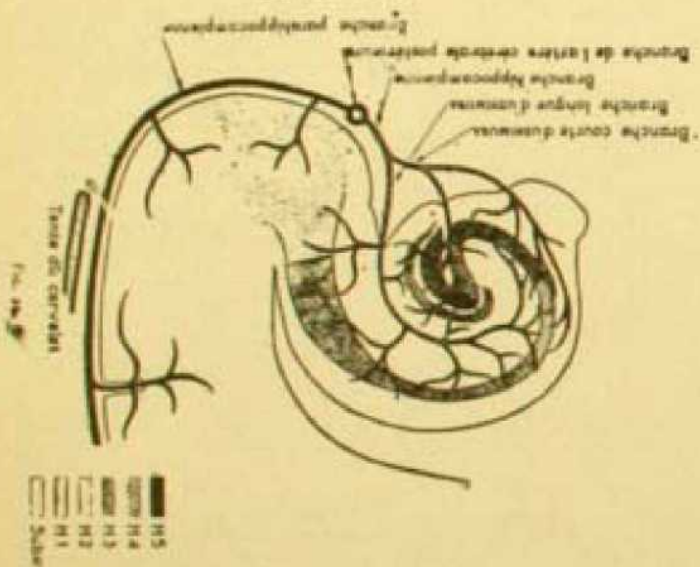


Fig. H 23

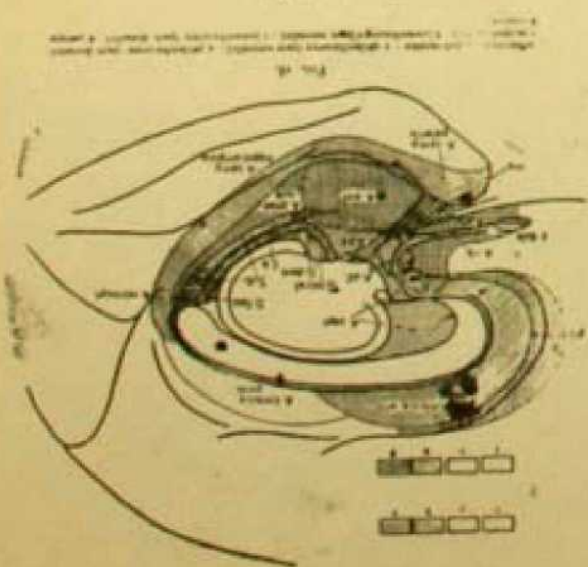


Fig. H 24

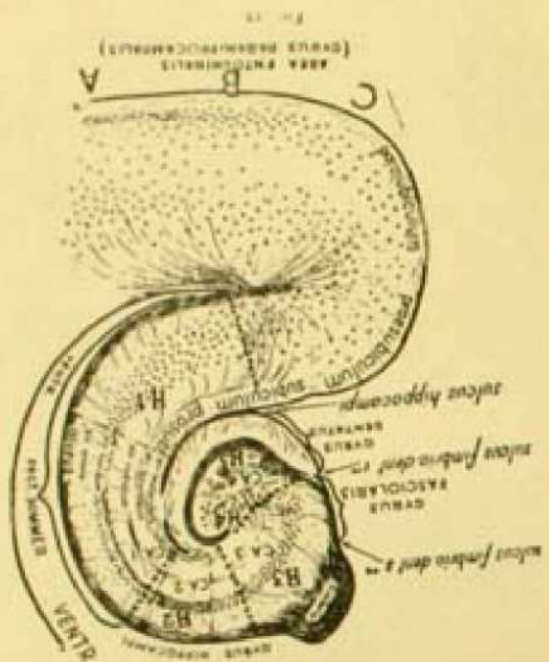
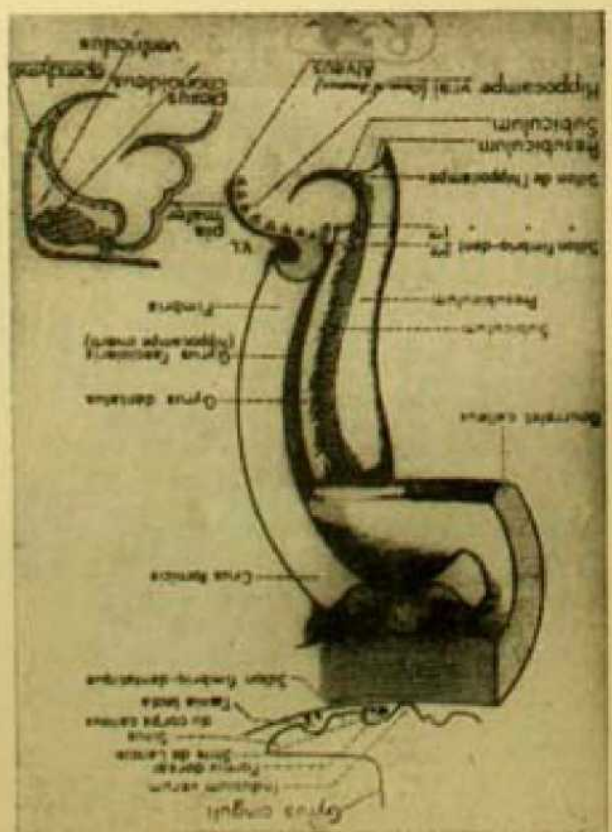
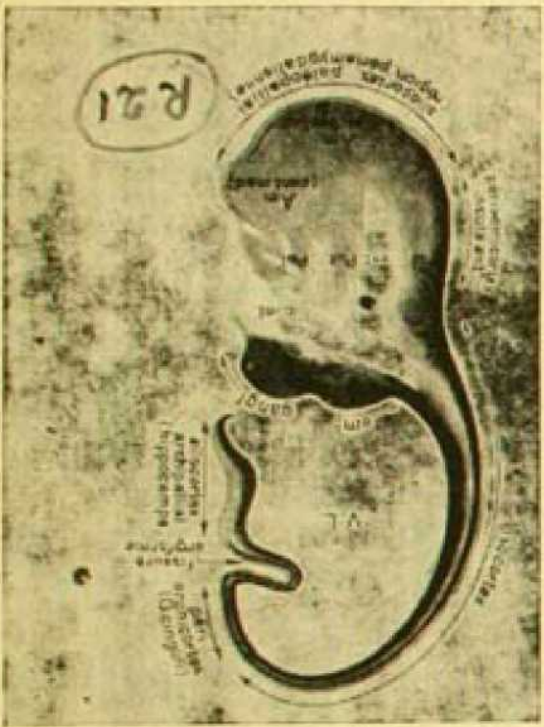
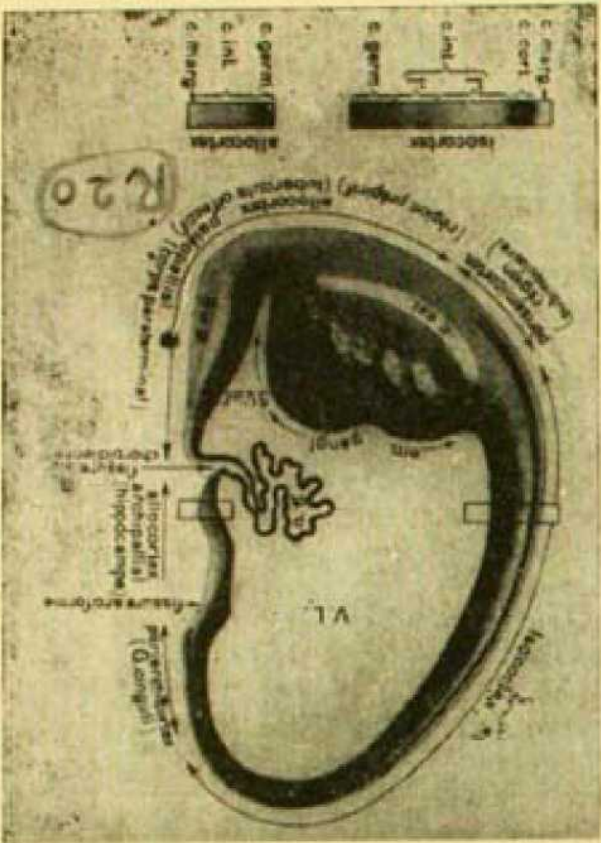


Fig. H 22

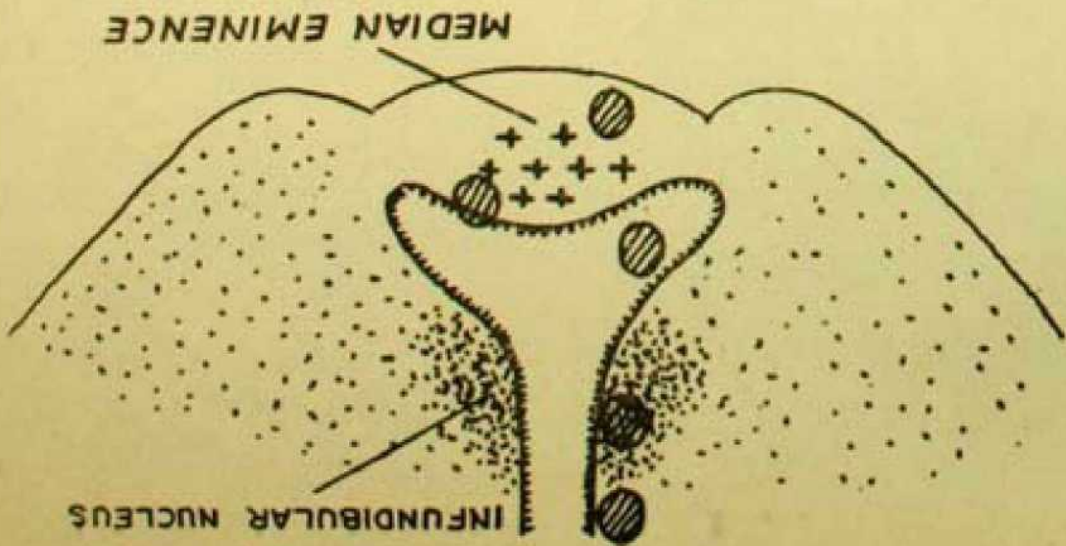






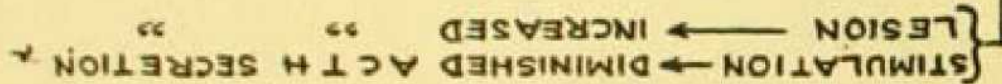
T. SECTION THROUGH MEDIAN EMINENCE  
OF C. versicolor SHOWING STIMULATED  
POINTS (+) & POSITION OF ACTIVE  
GRAFTS OF ANTERIOR PITUITARY (●)

Fig. 19



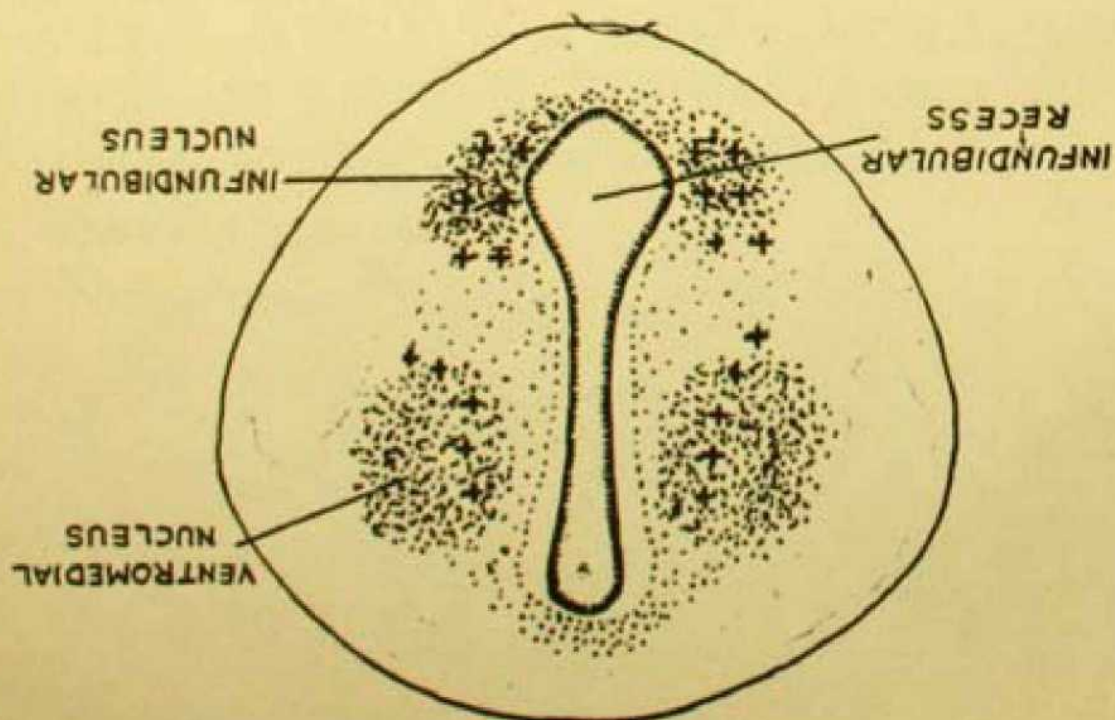


OF C. versicolor

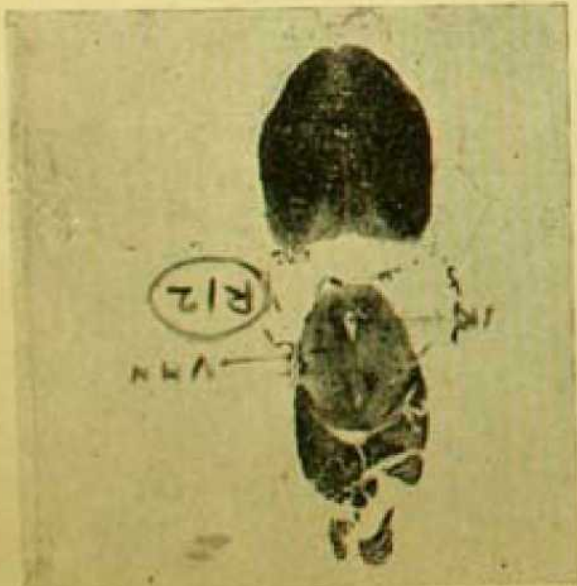
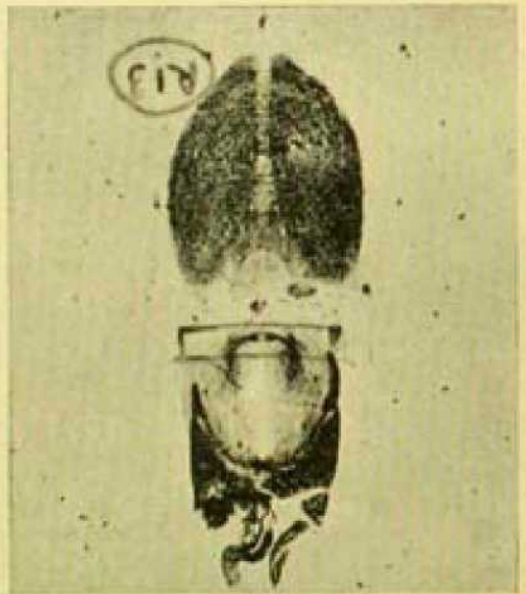
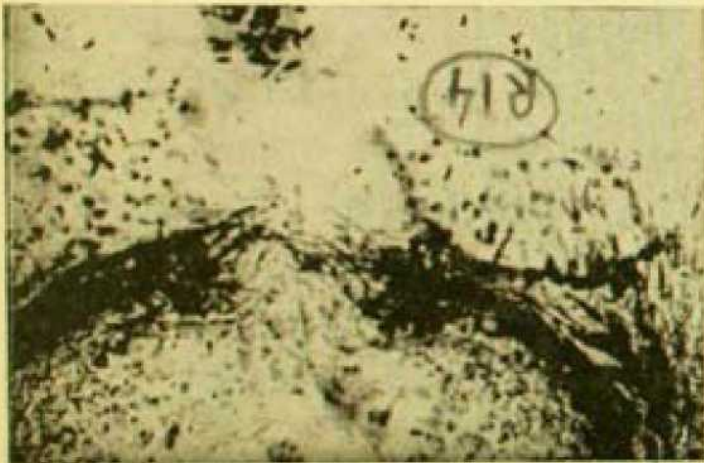
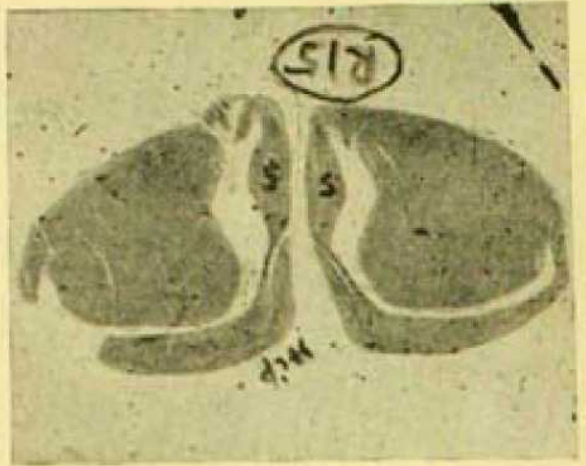
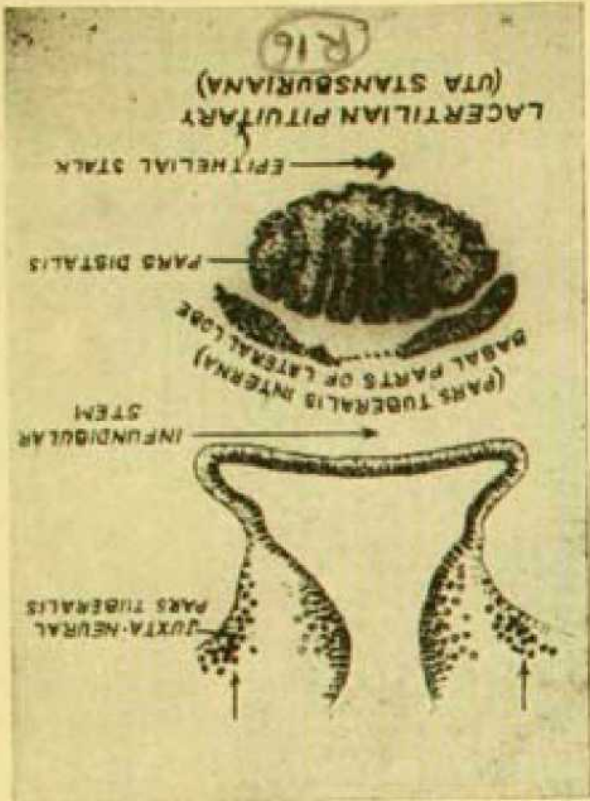


+ = STIMULATION OF POINTS → INCREASED BLOOD CORTICOSTERONE LEVEL  
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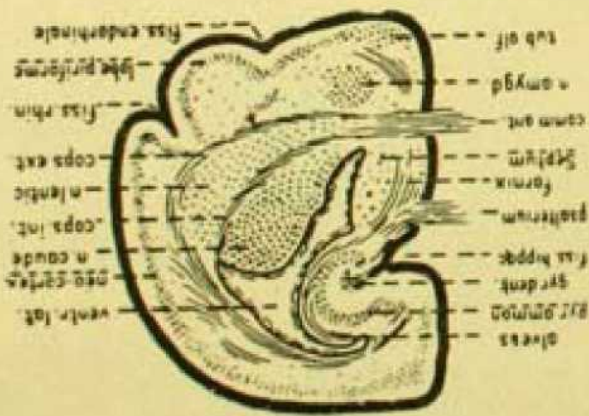
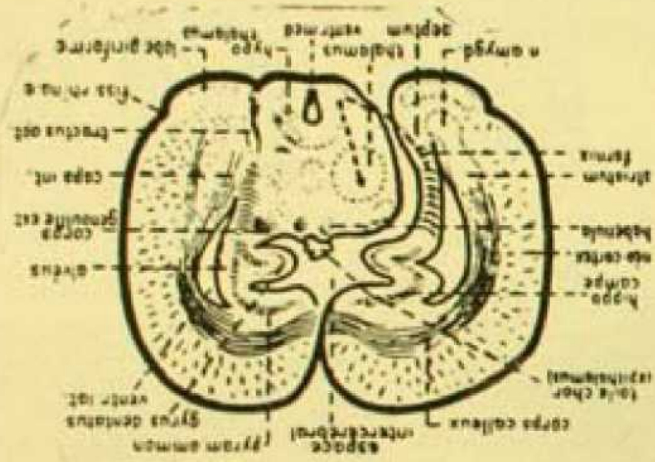
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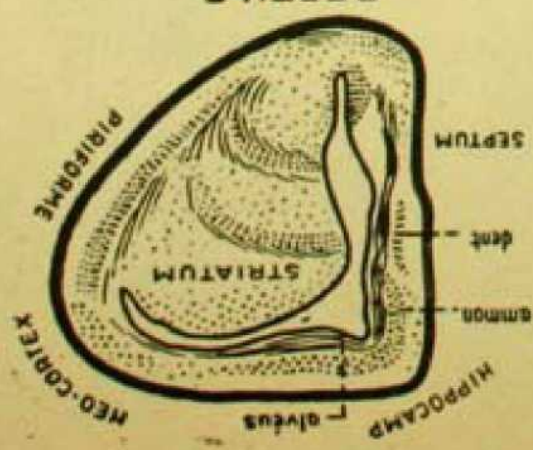








REPTILE



BATRACIEN

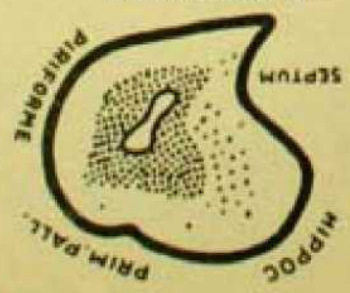
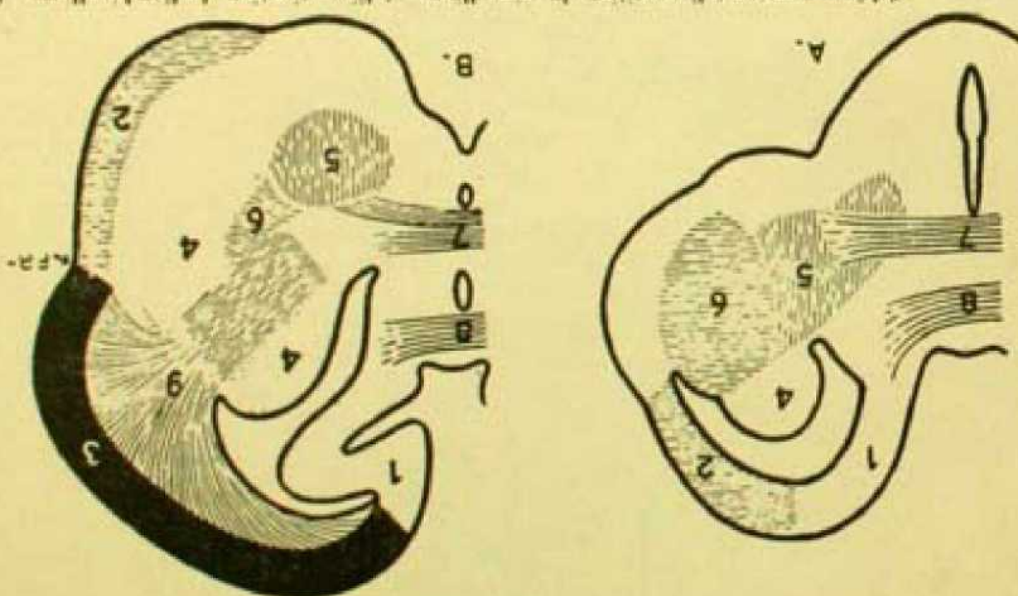




Fig. 1. *Diagramme* montrant l'évolution du développement du système nerveux central (SNC) chez le rat. Le diagramme illustre la progression de la neurogenèse, de la migration des cellules souches et de la différenciation des neurones. Les légendes indiquent : 1. Neurogenèse, 2. Migration des cellules souches, 3. Différenciation des neurones, 4. Formation des synapses, 5. Maturation du réseau neuronal.





— Chez les Poissons (fig. 2 A) l'ensemble du pallium est encore rhinocéphalique : archipallial en dedans, où il est représenté par le primordium hippocampi (p. hipp., en noir sur le schéma) ; palco-

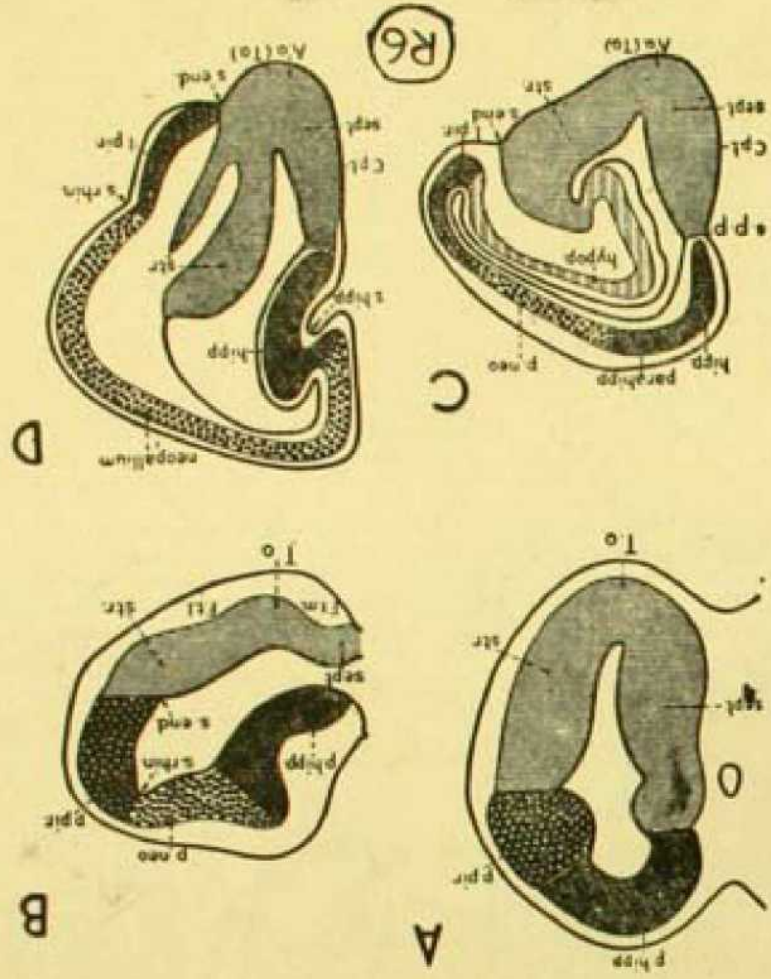


FIG. 2

(groupe sagittale d'après Kappers. Les éléments de l'anneau sagittal impair sont soulignés. Lèvre de la flexion basale.

— Cerveau de Légar.

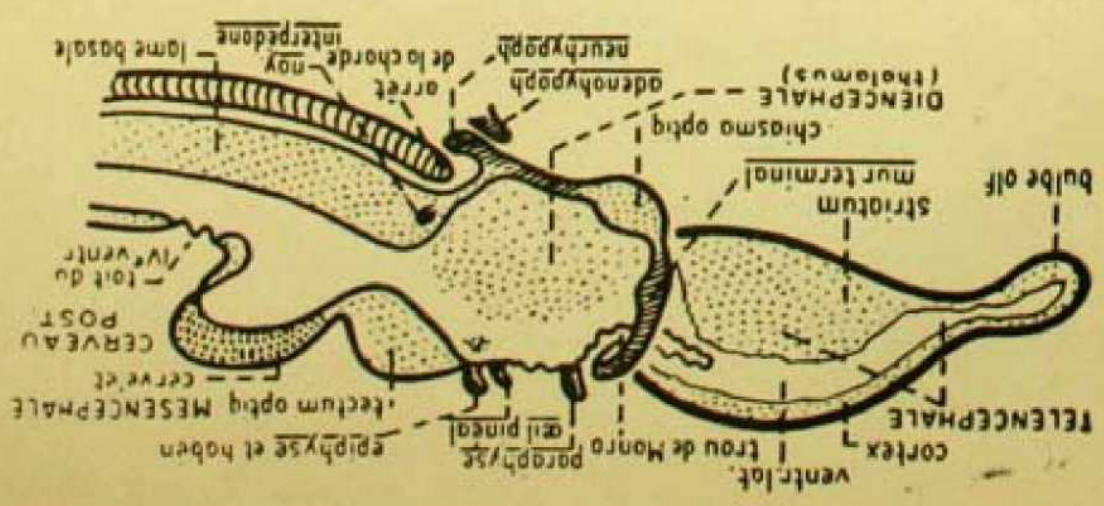




Fig. 11 4

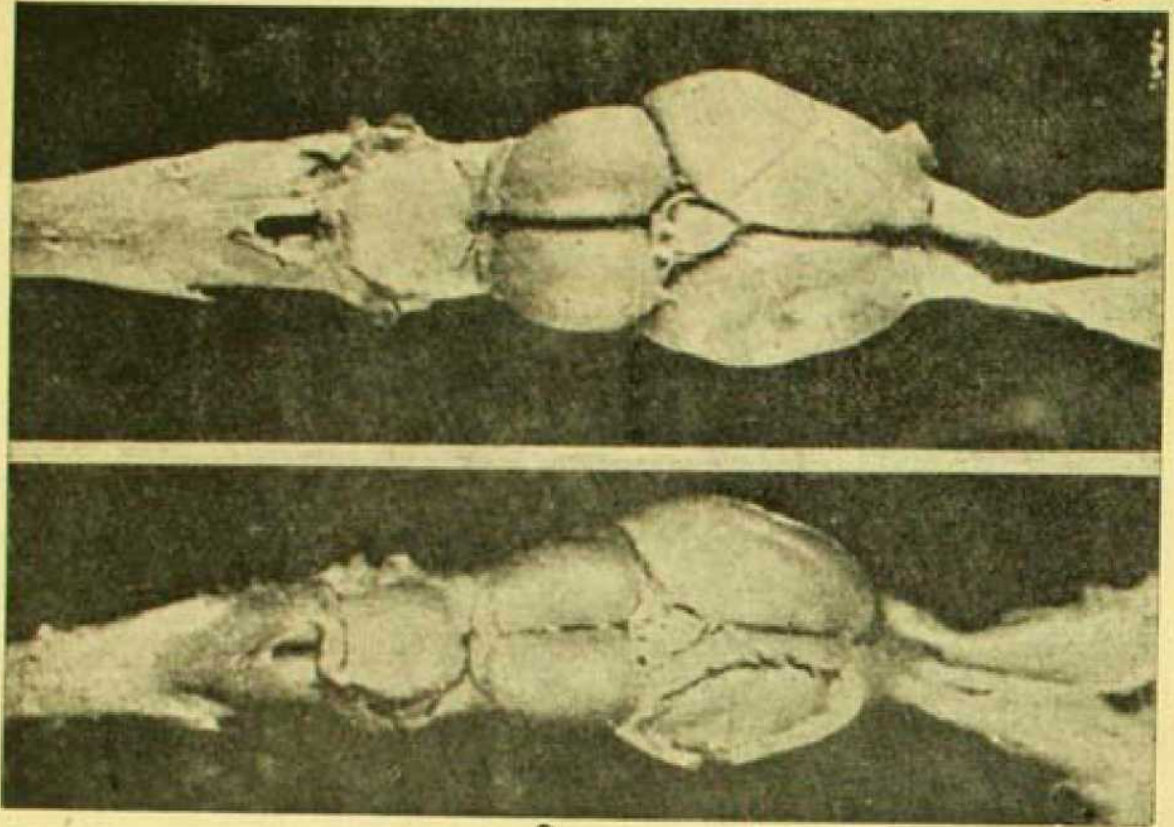
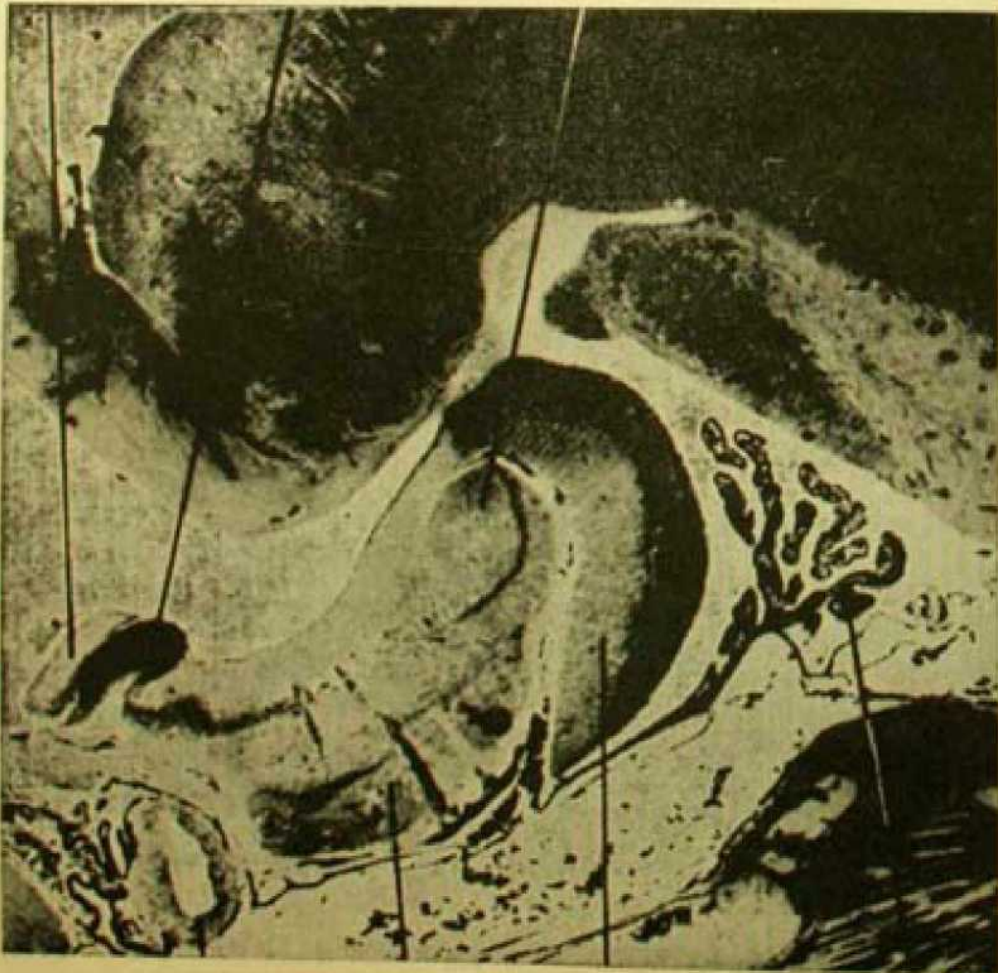
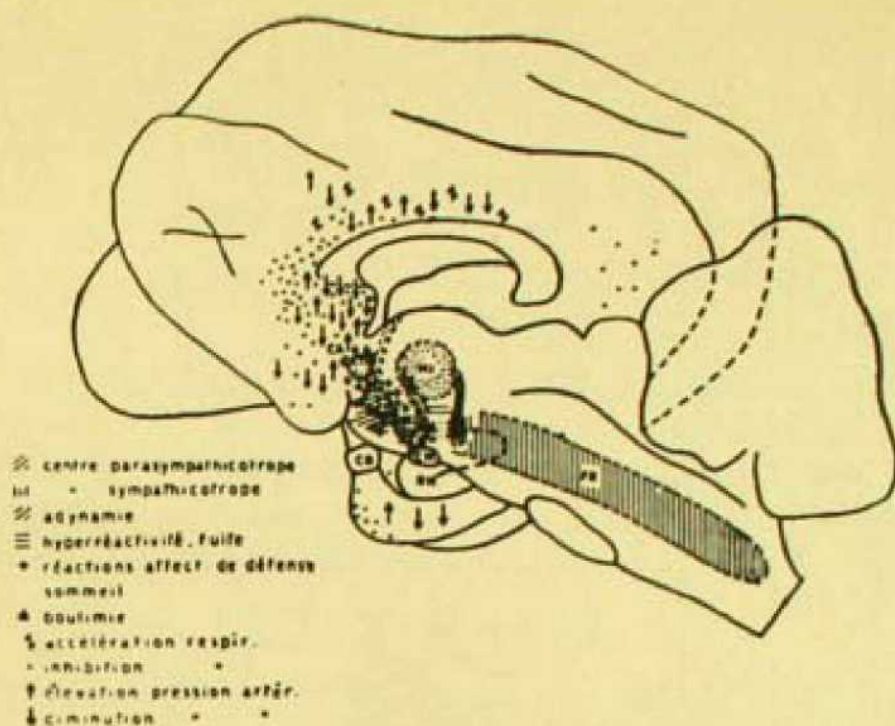


Fig. R 3







Centres régulateurs végétatifs du rhinencéphale (d'après Kaada, 1951)  
et du diencephale (d'après W. R. Hess, 1946).

FIG. R 44

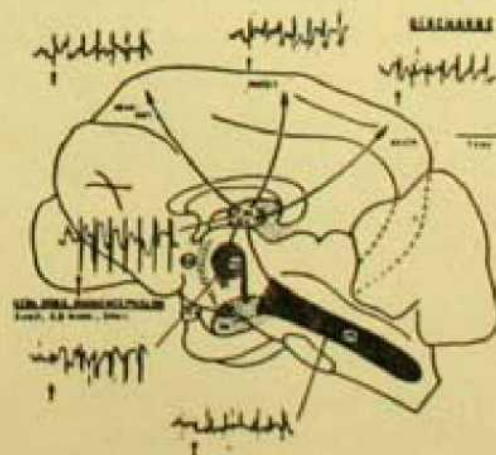


FIG. 4. — Répartition des réponses à la stimulation électrique (1.5 sec) de l'hippocampe (RH).

FIG. R 45



## CHAPTER 16

### THE HYPOTHALAMUS OF THE FISH

1975

Great variation in the development of the hypothalamus is noted in different vertebrate types. Greatest development is noted in teleosts amongst lower vertebrates. The hypothalamus is small in tailless amphibians. More differentiation is noted in reptiles. A high degree of development of the hypothalamus is reached in mammals (Kappers *et al.* 1967).

#### CYCLOSTOMES

The hypothalamus of cyclostomes is not highly developed. The recessus postopticus is behind the optic chiasma and caudally it is bounded by the commissura preinfundibularis. Then the infundibular region is noted. At the posterior end of the infundibular region a real saccus vasculosus is not formed because of the absence of neuroepithelium. The recessus hypophyseus is clearly present. The hypothalamus receives the tractus olfactohypothalamicus, the tractus thalamo-preoptico-infundibularis, the tractus tecto-lobaris and ascending fibres from the cerebellum. The infundibular regions of the two sides are connected by fibres which cross in the commissura preinfundibularis and the commissura postinfundibularis. Efferent tracts of the hypothalamus discharge to visceral centers of the medulla oblongata, specially the tractus lobobulbaris and the tractus mamillo-peduncularis. These tracts proceed caudalward along the nucleus interpeduncularis to the base of the medulla oblongata.

#### PLAGIOSTOMES

The ventral thalamus is separated from the hypothalamus by the sulcus thalamo-hypothalamicus internus. The hypothalamus is very large. It starts anteriorly near the preoptic recess. The optic chiasma is situated behind the recess and then there is a small postoptic recess. The infundibulum is separated from the postoptic recess by the Commissura preinfundibularis. The infundibulum is deep and bilateral recesses extend out from it and these are surrounded by massive nervous wall. These are called the *lobi inferiores* (Fig. 2). The thin basal plate shows two enlargements caudally—the brain-portion of the hypophysis and the saccus vasculosus behind it. The dorsal wall of the saccus passes on to the posterior wall of the *recessus mamillaris* and this is the caudalmost portion of the hypothalamus (posterior lobe of the hypothalamus) (Fig. 2).

The ventral thalamus and hypothalamus of the plagiostome receive the tractus strio-thalamicus et hypothalamicus. The uncrossed portion of the



tractus is large and the crossed portion is small. The decussation of the crossed portion takes place in the commissura anterior. Ventrolateral part of the telencephalon gives origin to the majority of the fibres. A part of the fibres also arises from septal regions. The fibres of this bundle terminate amongst the medium-sized cells in the ventral thalamic and hypothalamic areas. These cells do not group into distinct nuclei. They comprise the *nucleus strati grisei* forming a broad zone about one-fourth the thickness of the entire thalamus. The neurones are polygonal with an eccentric nucleus. The dendrites proceed in all directions from the cell body. The terminal portions of the striothalamic tract had synaptic relation with these cells. The neuraxes of the cells of the *nucleus strati grisei* proceed into the thalamo-tectal tract. Cells located along the lateral wall of the *lobi inferiores* and extending upward into the ventral thalamus constitute the *nucleus diffusus*. Situated further anteriorly is a special group of neurones which is called *nucleus interpeduncularis*. Primordium hippocampi and the hypothalamus are connected by the *tractus pallii* carrying ascending and descending fibres which are partly homolateral and partly contralateral. The *tractus medianus* connects the hypothalamus with the telencephalon (area superficialis basalis, and primordium hippocampi). This path was thought to be a homologue of the *fornix* of higher animals. Spino- and octavo- hypothalamic systems connect with more caudal areas of the hypothalamus.

The efferent fibres arise mainly in the lobus inferior and the recessus mammillaris. They form the *tractus lobo bulbaris* and the *tractus mamillo-peduncularis*. The *tractus lobo-cerebellaris* proceeds directly from the hypothalamus to the cerebellum. Other ascending fibres connect the hypothalamus with the septal areas.

The commissural systems are—commissura postoptica (commissura transversa), commissura preinfundibularis and commissura postinfundibularis.

The decussatio postinfundibularis inferior does not carry commissural fibres but crossed fibres of the *tractus saccivasculosi* are noted. The neuraxes of the neurosensory cells of the saccus vasculosus proceed to the gray substance of the tuberculum posterius and after decussating most fibres end around cells of the nucleus sacci vasculosi. Some fibres proceed to the central gray of the dorsal thalamus, constituting the *tractus sacco-thalamicus*. *Tractus tubero-posterior* also proceeds from the same nucleus. The *tractus thalamo-saccularis* takes origin in the anterior part of the dorsal thalamus.

#### GANOIDS AND TELEOSTS

The following nuclear groups have been noted amongst the hypothalamic (and preoptic) centers: nucleus preopticus magno-cellularis, nucleus tuberis lateralis, nucleus posterior thalami, nucleus tuberis anterior, nucleus tuberis



posterior, nucleus tuberis ventralis, corpus mamillare, ganglion sacci vasculosi, nucleus diffusus lobi inferioris and nucleus cerebellaris hypothalami.

Telencephalon influences the hypothalamus greatly. Connections are the *tractus olfacto-hypothalamicus medialis*, the *tractus olfacto-hypothalamicus lateralis* and the *tractus strio-thalamicus et hypothalamicus*. The *tractus olfacto-hypothalamicus medialis* starts from the pars supra-commissuralis septi and end in the lobi inferiores. *This represents the septal portion of the fornix system of higher vertebrates.* This tractus is associated with the *tractus hypothalamo-olfactorius medialis*. They together are some times called the *medial forebrain bundle*. This ascending tract starts from the nucleus tuberis posterior. Decussation of the fibres takes place in the diencephalon and in the commissura anterior. In pulses from the post-infundibular region proceed to the precommissural parts (septal) of the forebrain along this tract.

Lateral olfactohypothalamic and striohypothalamic tracts have both descending and ascending fibres.

Connections with dorsal thalamus are through the *tractus thalamo-lobaris* or *mamillaris*. The ascending fibres start from the large cells of the mamillary recess and lobi inferiores hypothalamici and are considered to be equivalent to the *mammillo-thalamic tract* or *Vicq d' Azer bundle of mammals*. Other connections are *tractus mesencephalo-lobaris* or *lobo-mesencephalicus*.

The *tractus tubero-dorsalis* connects the anteromedial part of the hypothalamus (*tuber cinereum*) with the *eminentia thalami*.

Further connection between the thalamus and the hypothalamus is made by the *tractus geniculo-hypothalamicus*.

Preoptic magnocellular nuclei are connected to the *tuber cinereum* by *tractus prethalamo-hypothalamicus*. This is also known as *fasciculus supraopticus* (unmyelinated fibres). At a caudal level this tract is joined by fibres from the ventral hypothalamic nuclei and passes into the hypophysis and the saccus.

The *tractus intralobaris* starts from the *tuber cinereum* and ends in the posterior part of the lobi inferiores. Connections between the ventral thalamus and the hypothalamus are made by the *tractus rotundo-lobaris* or *lobo-rotundus*.

There are also interrelating commissural systems, the *saccus vasculosus* and its connections, and connection with the lower centres.

#### AMPHIBIAN HYPOTHALAMUS

The posterior commissure marks the caudal boundary of the diencephalon dorsally. Anteriorly the floor of the diencephalon has a part of the chiasmal ridge having optic and supraoptic decussations. Posterior to this part, the



floor has the infundibular portion with hypophysis. In certain amphibians the junction between the diencephalon and the mesencephalon is called the tuberculum posterius.

Figures 3 to 7 show transverse sections through the tuber cinereum of frog behind optic chiasma, and in front of the hypophysis with points of stimulation (also in horizontal section).

Diepen (1962) on page 100 (Fig. 65) says that in the frog pars infundibularis adenohypophysis is noted. The primitive arrangement of the cells towards the ventricle (are periventricularis) is to be noted (Fig. 7). Division into medial and lateral fields is not seen. Lateral extension of the saccus infundibuli divides the tuber cinereum into dorsal and ventral portions. The periventricular cell area of the ventral portion contains the anlage of the nucleus ventromedialis and nucleus infundibularis of higher forms. In the dorsal portion, the anlage of the mamillary body is differentiated in the reptiles.

#### MEDIAN EMIENCE OR LIKE-STRUCTURE IN THE FISH MYXINIDAE

*Hypothalamo-neurohypophyseal* : neurosecretory pathway in Myxinidae was studied by Muller (1871), Retzius (1895) and Sterzi (1907). Recent publications include those of Adam (1956, 1957, 1959, 1963), Olsson (1959), Honma (1960) Gorbman (1965).

*The praeoptic nucleus Situation*: The groups of cells at the lateral border of the praeoptic recess in *Myxine* are thought to represent the praeoptic nucleus. The cellular products in these groups are not stainable by chrome haematoxylin, paraldehyde-fuchsin or astra blue (Adam, 1956; Olsson, 1959). A narrow band of ependymal cells is continued dorsocaudally from the ependyma of the praeoptic recess and these are apparently the remains of the more extensive ventricular lining of younger stages. This area therefore, is bounded dorsally by the primordium hippocampi, and anteroventrally by the nucleus praeopticus of Jansen (Fig. 1). The cells of the above mentioned area contain inclusions stainable by oxidized acid Astra blue. Positive reaction by the method described above is also noted in the beaded tractus praeoptico-infundibularis, the nsm of the infundibular process, the subcommissural apparatus, some ependymal cells and ventricular material. *The nucleus praeopticus* : The nsm is of small quantity and found as a fine granulation in the juxtannuclear position. These cells are situated at a distance from the ependyma and they do not send any dendritic processes to the ependyma. These cells always seem to be unipolar. This cell group is the homologue of *nucleus praeopticus magnocellularis* and the *nucleus praeopticus of Jansen* is the homologue of *nucleus parvocellularis anterior*. The latter group is nonneurosecretory (Olsson, 1959).

*Praeoptico infundibular tract* : Proximally this tract is not well discernible because the cell processes are not joined in a bundle. The formation into a bundle



occurs only distally at the median eminence region and the infundibular region. Secretory droplets are noted here and they are described as Herring bodies in other species.

*Infundibulum* : At the floor of the hypothalamic ventricle and in front of the infundibular process accumulations of neurosecretory material (nsm) are found. This is a characteristic region which has folded surfaces and well vascularized. Olsson (1959) tempts to compare this region in *Myxine* with the *eminencia mediana* of *Protopterus* and higher animals. Release of nsm into the vessels at this location takes place. However, Gorbman *et al.* (1963) considers this area as the most anterior part of the neural lobe or pars nervosa. Olsson (1959) noted that some of these blood vessels proceed ventralwards and reach the rostral cell cords of the adenohypophysis. The *second neurohaemal area* is located at the infundibular stem and release of secretory products into the bloodvessels at the surface of the brain takes place here also.

*The neural lobe* : The neural lobe in *Myxine* is represented by a saclike ventral prolongation of the hypothalamus where the fibres of the preopticoinfundibular tract end. The sac is flattened dorsoventrally. It has got a thick, vascular upper wall which is folded longitudinally. The floor of the sac is thin, less extensive and there are no folds.

The dorsal wall of the sac represents the neurohypophysis and the neurosecretory product accumulates mainly in it. It has got relationship with meningeal blood vessels and it represents a *third neurohaemal area*.

Olsson found a thick connective tissue septum separating the adenohypophysis on all sides from the infundibular process. Adam (1959) found openings in this capsule, more in the caudal and lateral parts, through which contact between adenohypophyseal tissue and neural lobe takes place.

#### ELASMOBRANCH

The axons of the preopticohypophyseal tract pass behind the optic chiasma in a diffuse way. In the caudal postoptic lamina the axons converge to form a discrete tract near the midline (Perks, 1969 ; Meurling, 1967). Neurosecretory granules and Herring bodies are noted in many fibres at this place. Here the portal vessels enter into the tract (Fig. 8). A median eminence region is thus formed (Meurling, 1960 ; Mellinger, 1960). Neurosecretory material has been noted near the blood vessels entering into the tract in *Scyliorhinus caniculus*. By light and electron microscope neurosecretory axons surrounded by glia cells have been noted to terminate on the capillaries. This area contains central mass of synaptic vesicles and plenty neurosecretory granules. It is probable that neurosecretory granules may pass from the terminals into the capillaries. The capillaries then drain into a complex of portal vessels which are the only blood supply of the rostral lobe of the adenohypophysis. In *Raja* and *Scyllium* an important portion



of the portal vessels passes to the neurointermediate lobe of the pituitary (Meurling, 1967) as is noted in the hagfish, *Myxine*. Wingstrand (1966) says that "This is unique among vertebrates as far as is known".

#### HOLOCEPHALIANS

The preopticohypophyseal tract is rich in neurosecretion and Herring bodies are noted. The tract runs posteriorly and at the level of nucleus lateralis tuberculi, loops of nerve fibres are given off which contain neurosecretion and have terminal swollen endings applied closely to the capillaries. The capillaries enter into the infundibular wall and they drain into portal vessels which supply the rostral part of the adenohypophysis. This part of the infundibular wall is considered by Sathyanesan (1965) and Jasinski and Gorbman (1966) as a *functional median eminence*.

#### PRIMITIVE BONY FISHES

*The Branchiopterygians*: Base of Rathke's pouch is retained in the adult as a duct connecting the oral roof with a cavity situated in the adenohypophysis. The neurohypophysis ramifies in the posterior part of the adenohypophysis and a saccus vasculosus is situated behind the neurohypophysis. Wingstrand (1966) states that in *Polypterus* "The pro-adenohypophysis is an elongate body of weakly staining cells, situated anteroventrally in the gland, and continues anteriorly with a thick, vascularized ligament which is attached to the preadenohypophyseal brain wall (Fig. 8). Wingstrand could confirm the presence of large saccus vasculosus and *neurohypophysis* having plenty of neurosecretory fibres in its branches. "The brain wall just in front of the *neurohypophysis* does not show particular differentiation, but further anteriorly, between and in front of the lobules of the meso-adenohypophysis, a *typical eminentia* was found in contact with the vascularized ligament described above. It has a typical palisade layer outside the thick, neurosecretory fibre bundles. Scattered granules of neurosecretory substance are seen in the palisade layer, close to the numerous capillaries which run in deep furrows on the surface. These capillaries communicate with the vessels in the vascular ligament, which probably carry the blood to the adenohypophysis. This latter point remains to be proved, however."

#### CHONDROSTEI

Polenov (1966) described a *broad neurosecretory contact area* in *Accipenser guldenstadt* Brandt or *A. stellatus* Pallas. The loosely arranged, nonmedullated neurosecretory fibres of the preopticohypophyseal tract send fine radial axons in the outer layer (Fig. 8). The axons end in club shaped, ribbonlike, or tapering terminal bulbs. They have contact with the vascular connective tissue separating the brain from the pituitary. The contact area is large having neurosecre-



tory material and no large Herring bodies are detected. The connective tissue membrane contains broad sinusoidal capillaries penetrating into the caudal part of the adenohypophysis. Polenov (1966) suggested that this contact area is the homolog of a *median eminence*.

### HOLOSTEI

Close association of neurosecretory material with the blood vessels penetrating into the wall of the infundibulum has been noted in *Amia calva*, *Lepidosteus ossens*, and *L. platostomus*. Herring bodies are seen to surround the capillaries. The vessels penetrate the connective tissue and enter into the rostral part of the adenohypophysis. A simple *median eminence* and pituitary portal system is thus formed (Dodd and Kerr, 1963; Sathuanesan and Chavin, 1967).

### TELEOSTS

Adult teleost pituitary consists of a compact adenohypophysis (pro-, meso-, and meta-adenohypophysis) and a neurohypophysis. The neurohypophysis is composed of solid branching processes of nervous tissue entering into the adenohypophysis. In some teleosts the hypothalamic floor is even with the adenohypophysis being attached to the neurohypophysis along its entire dorsal surface. This is called *platybasic type*. In others, the neurohypophyseal processes are given off from the top of a funnel like depression of the hypothalamic wall (hypophyseal stem). This is called *leptobasic type*. In *Lophius* the pituitary is situated much anterior to the brain and is connected with the hypothalamus by a long, narrow, nerve-like stem.

Diepen (1954, 1955, 1962) suggested that the anterior ramifications of the neurohypophysis consisting mainly of nonneurosecretory fibres should be regarded as a *modified median eminence* as they represent a form of proximal adeno-neurohypophyseal contact (Fig. 9).

In the eel, *Anguilla anguilla*, Leatherland *et al.* (1966), found that the pre-opticohypophyseal tract shows a small accumulation of neurosecretion caudally. Then there is a short empty area. This area was called *subterminal area* and it is situated above the anterior border of the adenohypophysis. This may represent the endings of certain neurosecretory axons. Perks (1969) stated that "It can only be concluded that the conventional pituitary portal system of the teleosts is at least anatomically underdeveloped and that it may even be absent."

The pars nervosa of *Mugil cephalus* was divided into two regions by Leray and Stahl (1961). The anterior part entered into the mesoadenohypophysis and the caudal portion ranified into pars intermedia. The anterior region had lesser neurosecretory material than the caudal one. In the rostral region the fibres came into contact with acidophil cells but in *Hippocampus guttulatus* these fibres passed to basophilic gonadotrophic cells (Leray and Stahl, 1961;



Da Lage, 1955, 1958). The less stained tracts in the rostral zone may come from the nucleus lateralis tuberis.

Ball and Baker (1969) differentiated two types of neurohypophyseal fibres depending on their stainability by AF, CAH, ATh, and AB. They are—*stainable fibres (probably Knowles' Type A)* and *nonstainable fibres (probably Knowles' type B)*. Neurosecretory material in the neurohypophyseal fibres may accumulate in large masses called Herring bodies.

Arginine vasotocin (AVT) and isotocin (IT) are two octapeptides found in the teleost pituitary (Sawyer, 1966).

From the works of Knowles and Vollrath (1966), Oliverreau (1967), Follenius (1963), 1965), it seems that the type A or type B fibres come into close approximation with all the adenohypophyseal cells (Ball and Baker, 1969). Most of the type A fibres go to the pars intermedia and most type B fibres pass into the pars distalis. Of course overlapping in distributions in each case is there. Type A fibres probably come from the nucleus preopticus and most type B fibres probably originate from the nucleus lateralis tuberis. The types of contact between the nerve fibres and the endocrine cells are (a) immediate physical contact, synaptic in some cases, (b) nerve fibres ending on a single or double basement membrane, and (c) nerve fibres ending in an extravascular space separating them from the endocrine cells.

Type A fibres are engaged with elaboration of peptide molecules and type fibres may secrete some nonpeptide material (aromatic amines).

Ball and Baker (1969) said that "Direct innervation of the pars intermedia is found throughout the vertebrates but the anterior neurohypophysis—pars distalis association is a teleostean speciality, and it may be that the extensive neurosecretory innervation of the pars distalis cells in this group evolved as an adjunct or supplement to the capillaries of the secondary centrifugal plexus. It is worth recalling the comparison, frequently made, between the median eminence of other gnathostomes and the anterior neurohypophyseal core of teleosts, a comparison all the more apt in the light of recent information, both being regions where hypothalamic neurosecretory fibres terminate on capillaries which convey blood to the pars distalis; it may be that the anterior capillaries of the secondary centrifugal plexus are functionally equivalent to the hypophyseal portal vessels, conveying products of neurosecretory cells (mainly type B) to the pars distalis, the median eminence and portal plexus being as it were enclosed within the pars distalis".

#### LUNGFISH

*Dipnoi*: Wingstrand (1966) says that the median eminence is situated along the anterior border of the pars distalis and it is like that of urodele amphi-



bians. The wall is thin, but there is a typical superficial palisade zone. The zone has furrows for the dense capillary net communicating with vessels of the pars distalis (fig. 10).

### MEDIAN EMINENCE OF ANURA

The median eminence is situated antero-inferiorly of the neural lobe (fig. 11) and having contact with the anterior end of the pars distalis. Three zones can be identified. The inner zone is lined by ependymal cells on the ventricular side. The middle zone has fibre bundles passing to the neural lobe. The external zone contains neurosecretory substance. Processes of glia cells (pituicytes) form palisade zones surrounding capillaries which drain by portal vessels into the pars distalis.

Budtz (1970) studied the ultrastructure of the median eminence of the toad, *Bufo bufo* after transections of the hypothalamus at different levels. In the zona externa of the toad median eminence there are five different types of neurones having different vesicles and granules. Less is known about their origin. Different hypothalamic cell groups give their origin. Budtz stated that all types of neurones ending in the zona externa of the median eminence of *Bufo bufo* take their origin from an area caudal to the preoptic nucleus but rostral and dorsal to the optic chiasma. Some of the type I—fibres (1000-1300 granules) appear to take their origin at a level below the optic chiasma.

### *Median eminence of Reptilia and Aves (figs. 12, 13)*

These have been discussed in previous chapters.

### *Hypothalamo-hypophyseal neurosecretory paths in man*

Spatz (1952) noted that the cells of the nerve fibres destined to the hypophysis in man are situated in that part of the hypothalamus where there are few or no myelinated fibres. The deficiency of myelin was thought by Spatz to indicate a low grade of ontogenetic and phylogenetic evolution. Connection with the hypophysis is made by supraopticohypophyseal and tubero-hypophyseal tracts and they come from different centres. The tubero-hypophyseal tract starts from hypothalamic nuclei close to the hypophysis (*hypophysennahen Anteile des markarmen Hypothalamus*) (Spatz, 1958 ; Diepen, 1962). The nuclear groups are the infundibular (nucleus arcuatus), ventromedial, dorsomedial nucleus and those of the posterior periventricular area. Delicate unmyelinated fibres come from these cell groups in the tuber cinereum and end around the infundibular vessels. Connections of the tuberomammillary nucleus and the lateral nucleus of the tuber with the hypophysis are debatable. In teleosts the lateral nucleus of the tuber shows secretory cycle. Spatz says that the supraoptic and paraventricular nuclei giving rise to supraoptico-hypophyseal tract are situated in a part of the hypo-



thalamus remote from the hypophysis (*hypophysenferne Anteile des markarmen Hypothalamus*).

The median eminence is situated in the floor of the infundibulum and it is highly vascular. Intimate contact between nervous and glandular tissues with accumulations of neurosecretory product takes place here (Gabe, 1966). Spatz suggested that this area should be called *suprasellar hypophysis*.

#### MEDIAN EMINENCE OF THE RAT

Rinne (1970) noted that in the median eminence dopamine is not contained in the granular vesicles which are made visible by glutaraldehyde and osmium. Small granular vesicles may be involved in the storage or metabolism of noradrenaline in the median eminence. From the experiments of bilateral adrenalectomy it could be found that the aldehyde-fuchsin-staining substance and the large granular vesicles may be connected with the neurohumoral control of corticotrophin secretion. No evidence could be found which indicated that dopamine plays a role in this process. "The large granular vesicles may act as the storage site of *corticotrophin-releasing factor* and may be the subcellular structures corresponding to the aldehyde-fuchsin-staining substance in the median eminence. Obviously the increased number of large granular vesicles found in the present study corresponds to the abundance of large empty vesicles described by Akmayev *et al.* (1967) 10 to 17 days after bilateral adrenalectomy."

Raisman and Field (1971) schematically represented the ependymal modifications in the infundibular recess of the third ventricle and the principal relationships in the median eminence of the rat (fig. 14). There are ciliated ependymal cells and in the floor of the infundibular recess there are smooth, bricklike ependymal cells. In the median eminence there is an internal zone, an external zone and in between the two zones there are fibres of the hypothalamoposthypophyseal tracts. The tanycyte ependymal cell has got a process which is directed into the anterior part of the median eminence having contact with the fenestrated capillaries opposite the agranular cells of the pars tuberalis. The arcuate neurone has an axon which terminates in the main part of the median eminence together with terminals of pituicytes—both contacting the basement membrane associated with portal capillaries facing the granular cells of the pars tuberalis.

Stimulated points of the hypothalamus of guinea pig leading to increase ACTH release are shown in figure 15.

Calas *et al.* (1974) studied the indolaminergic fibres in the median eminence of the duck, rat and monkey. The results demonstrated the existence of indolaminergic fibres in the median eminence. In the bovine and duck median eminence there is existence of structures containing 5-HT. That the indolaminergic fibres are truly serotonergic can be said only when they can be identified with structures containing 5-HT.





Clearly defined-synaptic junctions are absent in the indolaminergic fibres. Their proximity to the processes of tanyocytes and the external basement membrane indicates that they may be involved in the transport of hypothalamic factors by glial cells and/or their release into the portal vessels.

Stutinsky (1970) said that at least three types of vesicles could be found in the median eminence of the rat and the guinea pig. 1 Neurosecretory endings having synaptic vesicles and elementary granules with a diameter of 2000 Å. The dense cores completely filling up the vesicles are in close contact with the membrane. (2) Nerve endings with synaptic vesicles and a few, small, dense-core granules in vesicles from about 600 Å to 900 Å having a definite external membrane. (3) Nerve endings with synaptic vesicles very similar to the neurosecretory elements but of a smaller size (from 800 Å to 1200 Å).

*Secretory cycle and cell structures responsible for the formation of secretory product in neurosecretory cells.*

Please refer to previous chapter.

#### *The transport hypothesis*

Neurosecretory product migrates proximodistally along axons. Gabe (1966) said "Actual evidence now available on displacements of substance within the nerve cells, evidence which has recently been reviewed by Weiss (1961), supplies a solid physiological basis for the *Transport hypothesis* of Scharrer, which has been adopted and developed by Bargmann and is now accepted by most authors". This concept postulates that the active substances and the carrier neurosecretory product pass out of the cells in completely formed stage and no transformation occurs during axonal migration. The opponents of this concept think that elaboration of neurosecretory product takes place in the axons and terminations of the neurosecretory cells. Gabe (1966) stated that "In the present state of our knowledge there is no decisive argument supporting the genesis of neurosecretory product *in situ*, but the theory that it may undergo modification in the course of axonal migration cannot be discarded out of hand".

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## Chapter 16

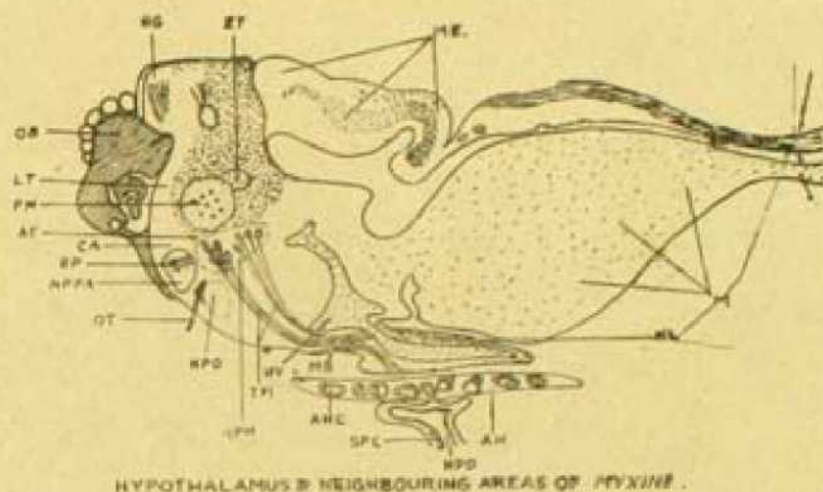
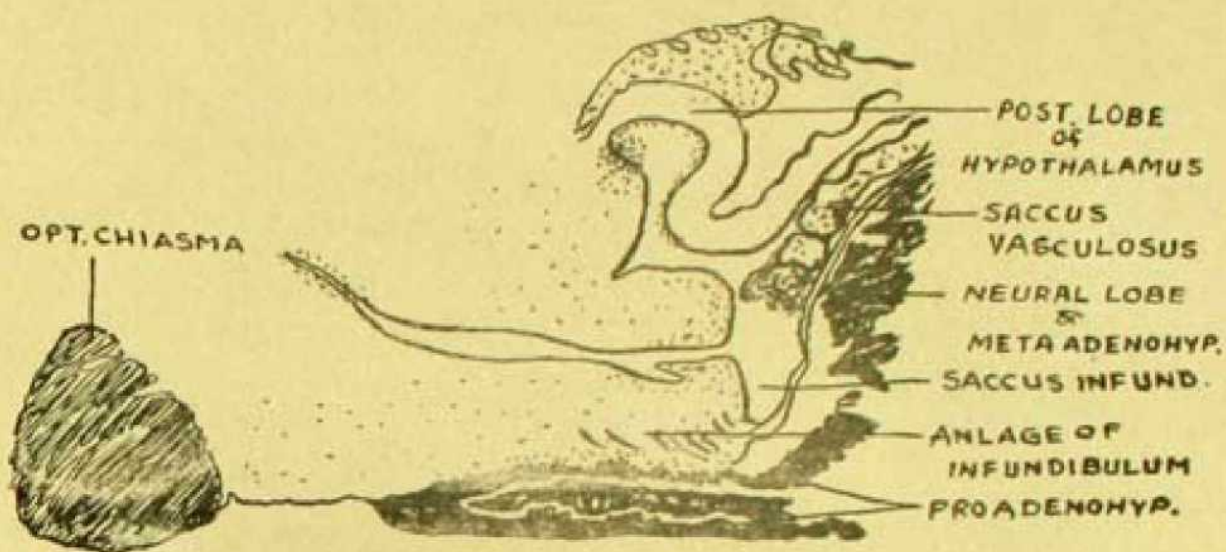


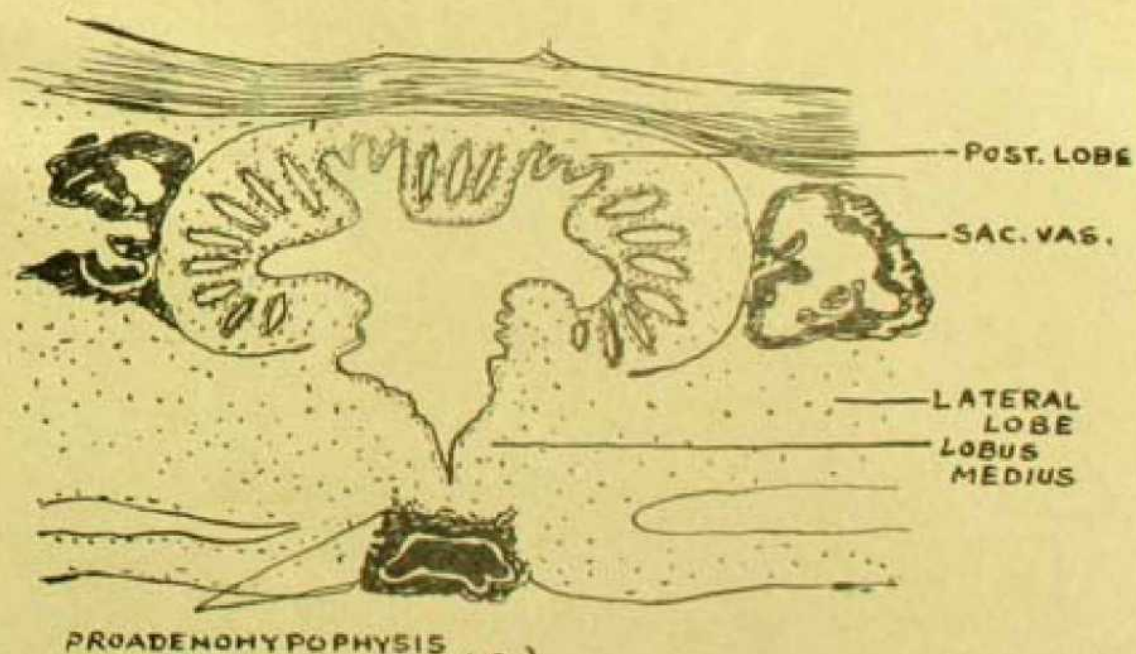
Fig. 1. The hypothalamus and neighbouring areas of *Myxine*.

- HG —Habenular ganglion
- OB —Olfactory bulb
- LT —Lamina terminalis
- PM —Primordium hippocampi
- CA —Commissura anterior
- RP —Recessus preopticus
- NPPA —Nc. preopticus parvocellularis anterior
- OT —Optic tract (poorly developed)
- NPO —Nucleus postopticus
- NPM —Nucleus preopticus magnocellularis
- TPI —Tractus praeoptico-infundibularis
- HV —Hypothalamus ventricle
- AHC —Adenohypophyseal cell
- SPC —Spider cells
- NP —Ductus nasopharyngicus
- AH —Adenohypophysis
- NL —Infundibular process
- Me —Mesencephalon
- ME —Median eminence
- M —Moelle



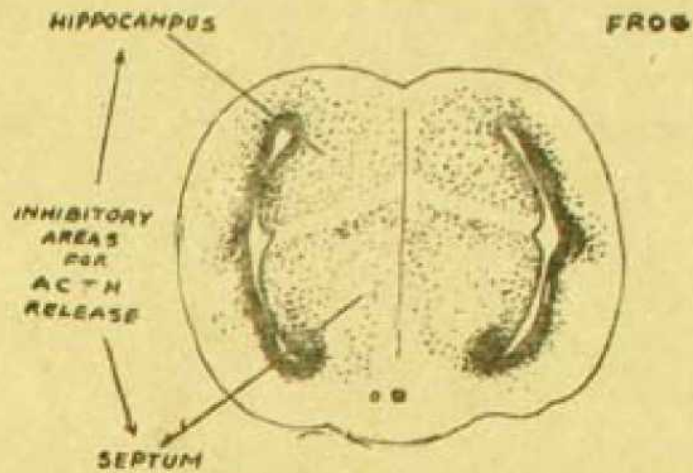


SAGITTAL SECTION OF THE HYPOTHALAMUS OF SHARK.  
(A)



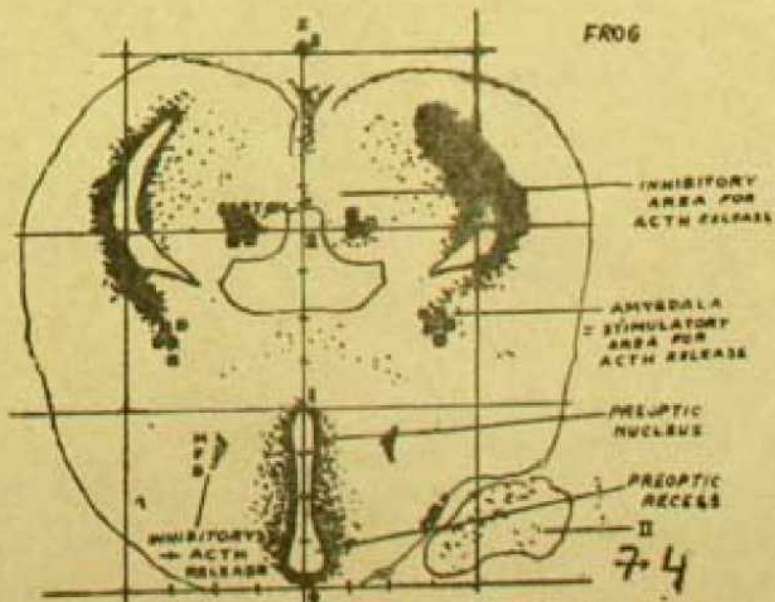
TRANSVERSE SECTION THROUGH THE POST. LOBE  
OF THE HYPOTHALAMUS OF THE SHARK.  
(After Diepen).



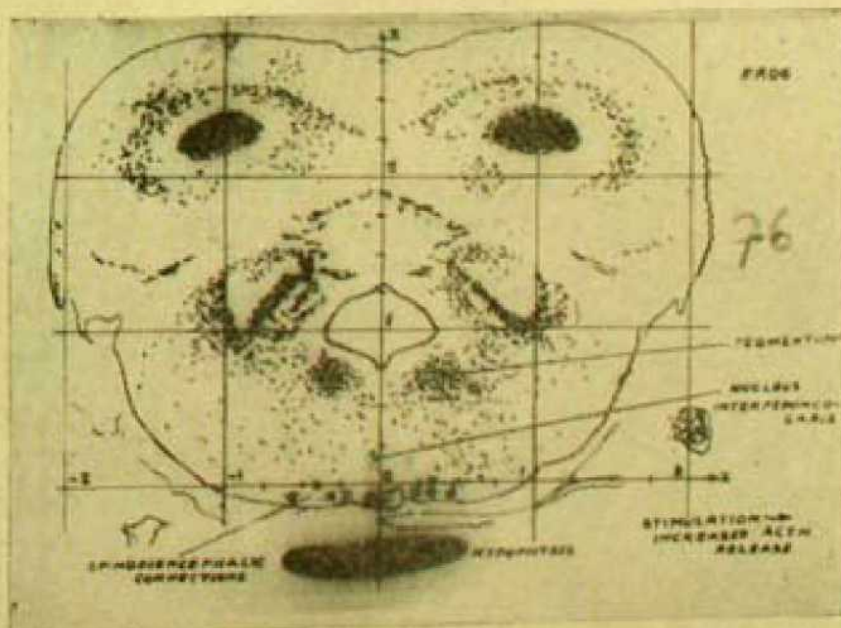
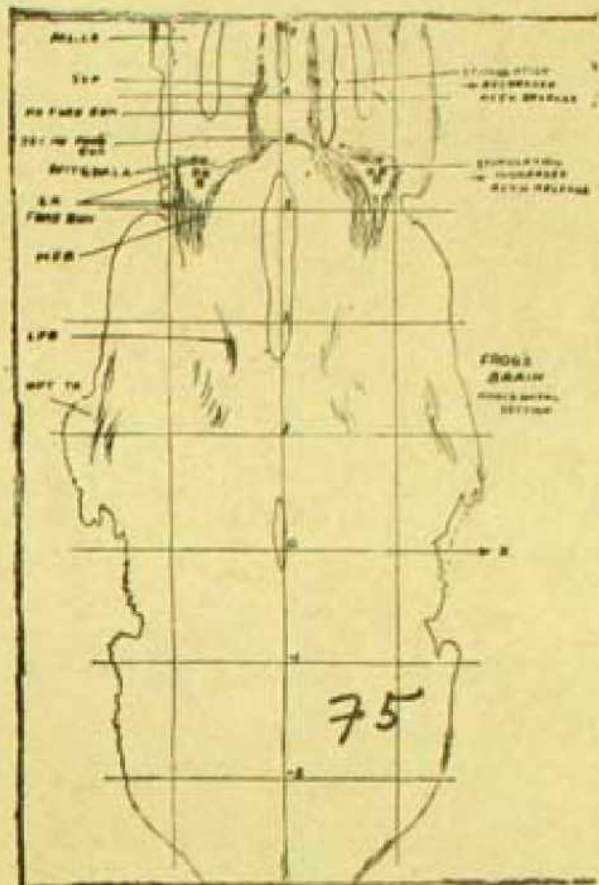


TR. SECTION THROUGH THE  
TELENCEPHALON

Fig. 3









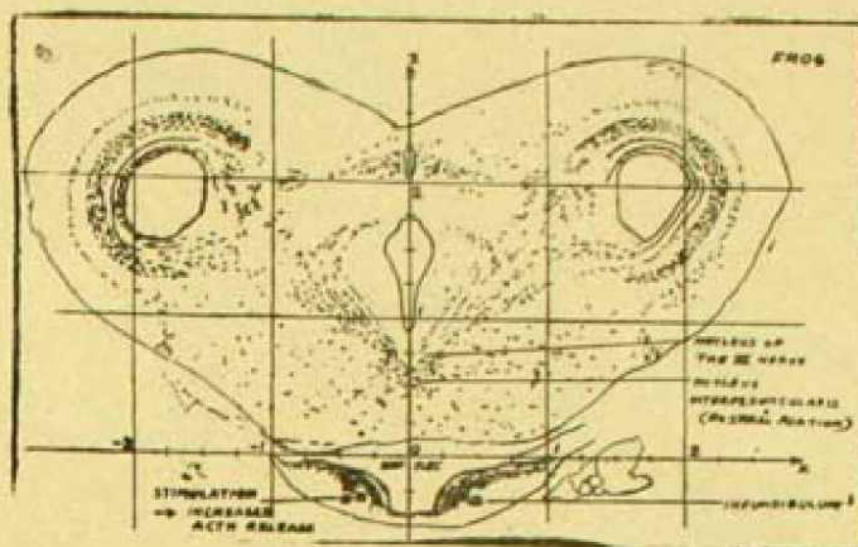


FIG. 7.

Figs. 3-7. Sections showing points of stimulation of the brain of the frog leading to increased or decreased ACTH release.

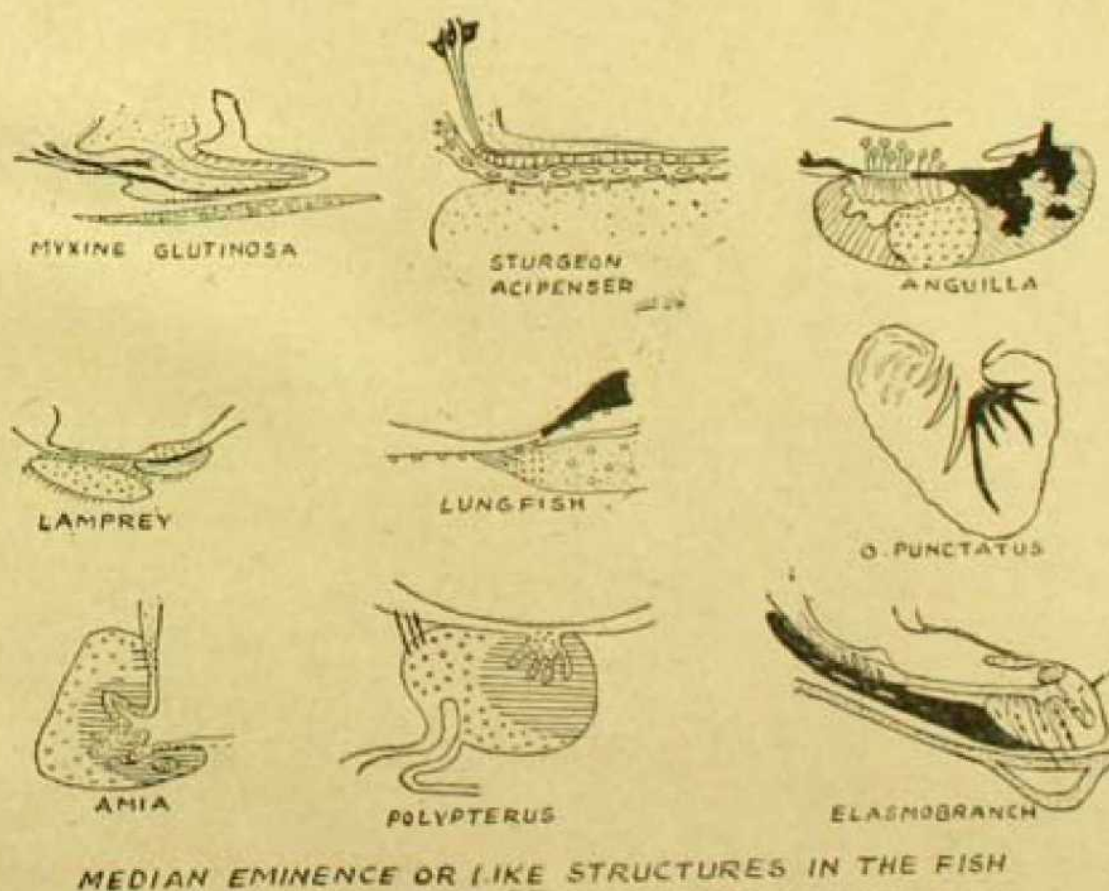
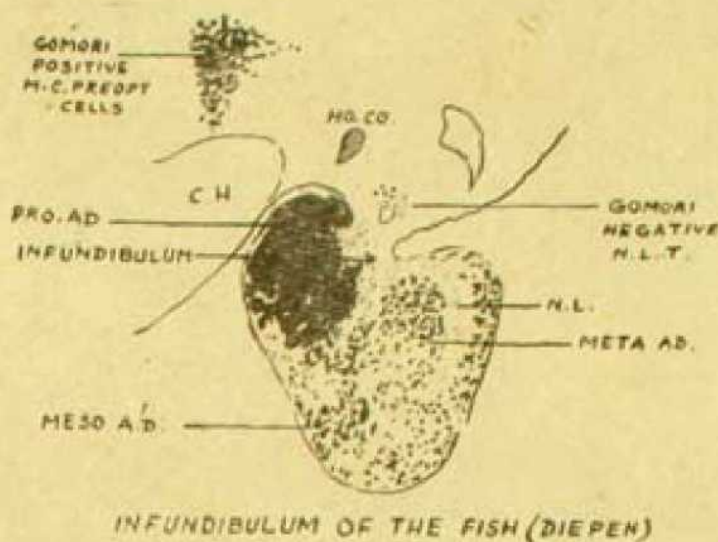


Fig. 8. Median eminence or like-structures in the fish.





INFUNDIBULUM OF THE FISH (DIEPEN)

Fig. 9. Infundibulum of the fish (Diepen).

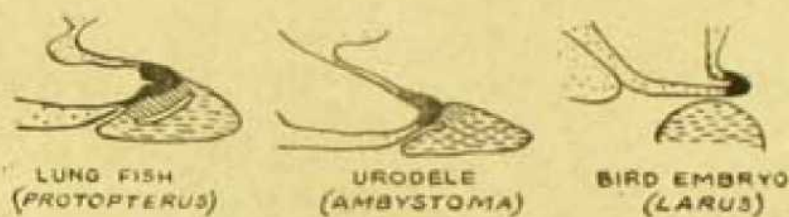


Fig. 10. Median eminence of the lungfish (Wingstrand).

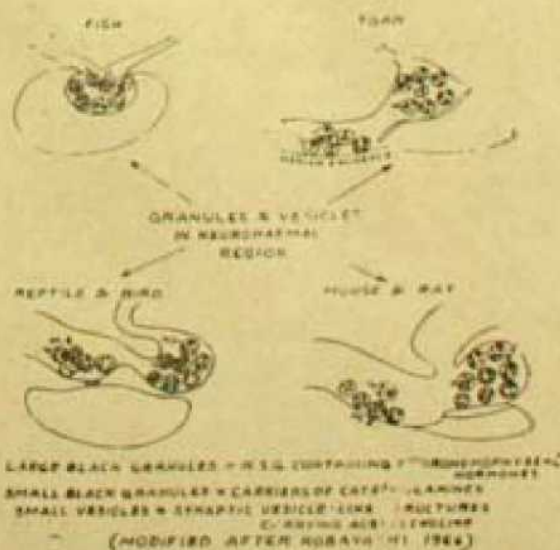


Fig. 11. Granules and vesicles in the neurohaemal region of the fish, toad, reptile and bird, and mouse and rat (After Kobayashi, 1964).



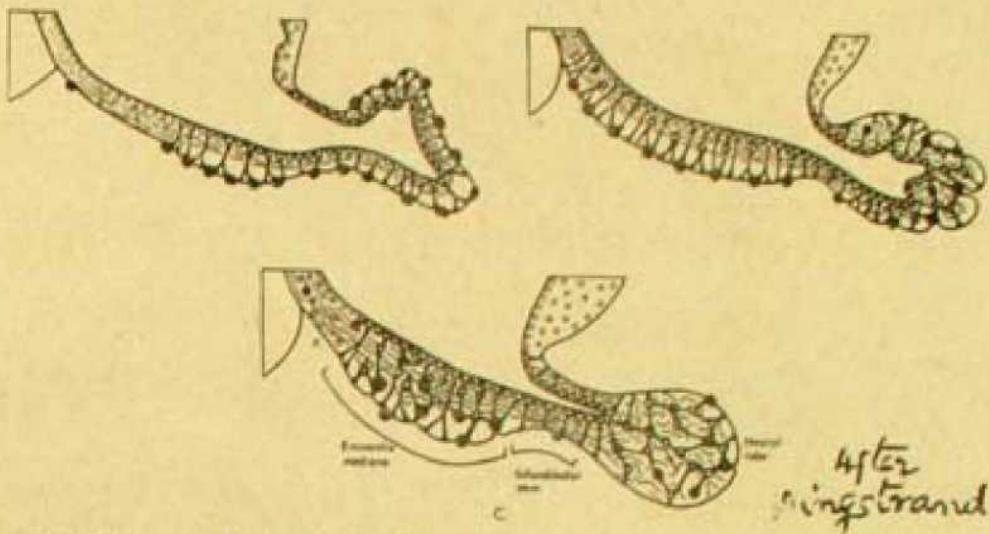
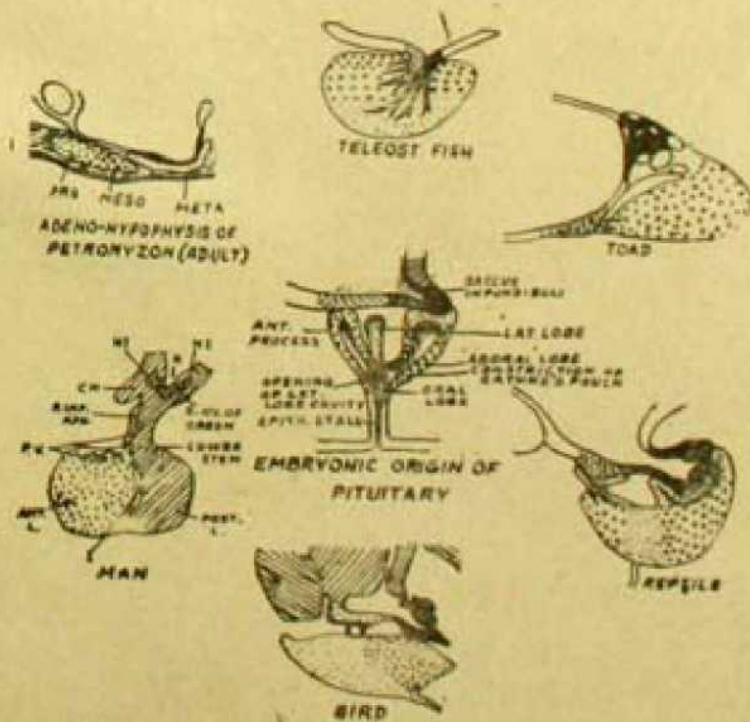


Fig. 12. Diagrams showing the histological differentiation of the neurohypophysis in amniotes. In A a primitive structure (most Lacertilia, *Sphenodon*), in B the structure in the majority of birds, in C the mammalian type. The processes of ependymal cells, pituitary cells and glia cells are stippled, the blood vessels are black, and the coarse nerve fibres are indicated by lines. Connective tissue elements are not considered. X = pars oralis tuberculi.



Figs. 12, 13. Median eminence of reptilia and aves (After Wingstrand).



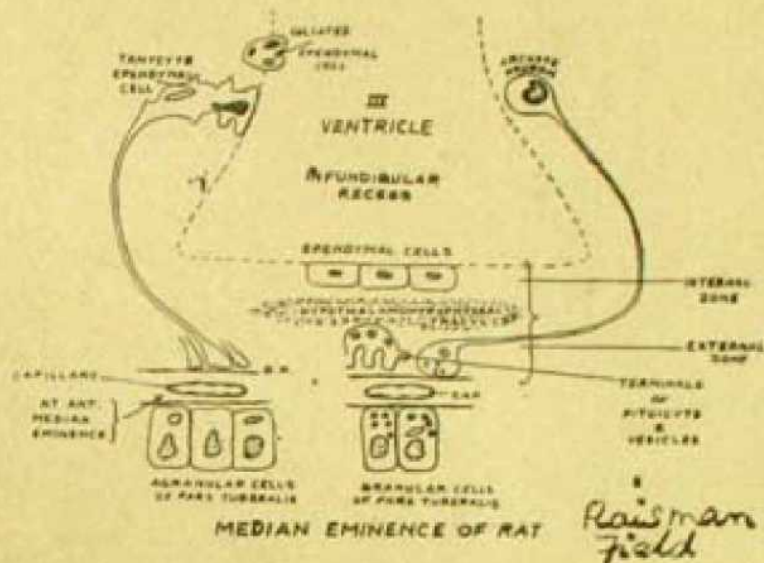


Fig. 14. Median eminence of the rat (After Raisman and Field, 1971).

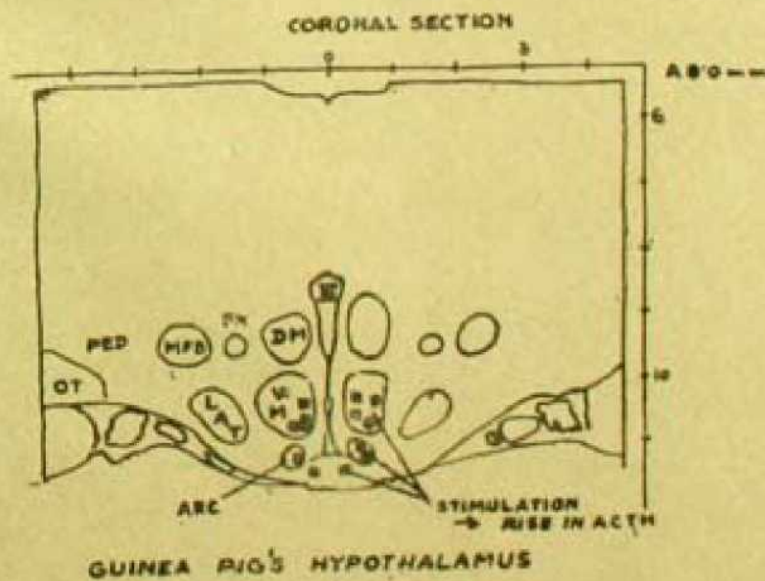


Fig. 15. Shows the points of stimulation in the hypothalamus of the guinea pig leading to increased ACTH secretion.

VM — Ventromedial nucleus  
ARC — Arcuate nucleus





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## CHAPTER 17

### HYPOTHALAMO-PITUITARY-ADRENAL-AXIS OF BIRDS (1972-1974)

#### HYPOTHALAMUS, HYPOPHYSEOPORTAL VESSELS AND HYPOPHYSIS OF BIRDS

##### *Hypophyseoportals vessels in the birds :*

The importance of the hypophyseoportals vessels in the control of the anterior pituitary function has been stated in previous chapters. Green (1951) and Hasegawa (1956) described these vessels in the chicken. Wingstrand (1951) investigated them in the pigeon and other birds. Assenmacher (1952, 1953, 1958) described these vessels in the duck in details.

Benoit (1962) discussed the importance of the hypothalamic control over the adeno-hypophyseal gonadotropic function through the hypothalamo-hypophyseal connections in ducks. There is complete involution of the gonads when the connections are divided. Assenmacher (1958) found the thyrotropic and corticotropic functions to be little affected after division of portal vessels or lesions of the median eminence or the hypothalamic magnocellular nuclei. Ectopic adeno-hypophyseal grafts could not activate the gonads. When the adeno-hypophysis does not get special blood supply from the median eminence, it cannot maintain normal gonadotropic function. When the infundibular stalk is divided, there is complete atrophy of the posterior lobe ; but the gonadotropic function is undisturbed because the median eminence, the portal system and the adeno-hypophysis remain intact. Lesion in the median eminence leads to complete and permanent atrophy of the gonads. Bilateral lesions of the anterior hypothalamic regions (supraoptic and paraventricular) is followed by genital atrophy (2-3 weeks). Testes depend completely on intact hypothalamo-hypophyseal connection, the thyroids depend less and the adrenals the least.

Vitums *et al.* (1964) described the vascularization of the hypothalamo-hypophyseal complex in the white-crowned sparrow, *Zonotrichia leucophrys gambelii*.

Preoptic arteries which are branches of right and left anterior cerebral arteries supply the supraoptic and paraventricular nuclei. The median eminence and the neural lobe get their supply from the infundibular artery. For the anterior and posterior divisions of the median eminence there are anterior and posterior capillary plexuses. Anterior and posterior groups of portal vessels are formed from the plexus and they are distributed to cephalic lobe and caudal lobe respectively.

Wingstrand (1951) studied the vascular supply of the avian pituitary (Fig. 1) and in general his investigation corroborated that of Green (1951). The neural lobe has got an independent blood supply (Fig. 1)



from arterial branches either from infundibular arteries or they are true inferior hypophyseal arteries from intercarotic anastomosis. The venous drainage from the neural lobe is to the sinus cavernosus. The *primary plexus* of the eminentia is supplied by the infundibular arteries. The drainage from this plexus is by the portal vessels contained in the portal zone of the pars tuberalis and passes to the *secondary plexus* in the pars distalis. The cavernous sinus drains the pars distalis.

A dense capillary net covers the median eminence on the surface or sinks deep into the furrows of the median eminence as in *Anser anser*. This capillary net extends to the surface of the infundibular stem but the density here is low. The capillary bed of the neural lobe is independent of this system. The primary plexus is also isolated from the hypothalamic vascular bed. The hypothalamic capillaries are subependymal in position. The nucleus tuberis is supplied by these capillaries.

The blood passes from the median eminence to the pars distalis through the portal vessels and not in the opposite direction. This has been stated by Wingstrand (1951). Transport of secretory products can take place from the median eminence to the pars distalis *via* the portal vessels.

Szentágothai *et al.* (1968) extended the work of Török regarding the direction of blood flow in the portal vessels of the dog, cat and rat. The pars distalis is supplied through portal vessels arising from a *primary plexus*, that is, plexus of the pars tuberalis and median eminence capillary loops contribute to the supply. The passage of blood is from *pars tuberalis*—median eminence—portal vessels—anterior lobe (pars distalis) tissue. There are two other possible routes of blood flow having functional significance. "(i) A portion of blood, after passing the pars tuberalis and entering the capillary loops of the median eminence, is drained over the interior median eminence plexus towards the medial (subependymal) capillary network of the hypothalamus. (ii) Some of the blood, reaching the posterior surface of the anterior lobe in the pars distalis sinusoids, turns upwards and is drained over the anterior transition zone (*Umschlagszone*) between pars distalis and pars intermedia towards the posterior lobe vascular system. (iii) It is even possible that part of the blood taking route (ii) might find its way into the interior plexus of the infundibular stem and is finally drained towards the hypothalamus (i)."

Pars tuberalis is a less differentiated structure than the pars distalis but it contains some amount of tropic hormones and clearly shows signs of secretory activity (PAS-positive and Bodian-positive granules). Blood coming to the pars distalis gets a contact first with the pars tuberalis and then with the median eminence and thus the median eminence is being influenced. Some fraction of this blood reaches the median hypothalamus which is being influenced by substances produced in the pars distalis. Szentágothai *et al.* (1968) say that this is a *mechanism of biologically important signal transmission*.

The pars tuberalis of birds consists of (1) the *pars tuberalis proper*, which is constituted by a thin layer of cells lying within the pia mater on



the surface of the brain, and (2) a *portal zone* having strings of epithelial cells connecting the pars tuberalis proper with the pars distalis. The portal zone is continuous with (3) the paired *pars tuberalis interna* which fuses intimately with the pars distalis (Wingstrand, 1951). In the *Procellariiformes* the pars tuberalis has a very characteristic form and is not a vestigial organ. The pars tuberalis proper is very thick and in its central parts there are several layers of epithelial strings and a rich capillary net. The portal zone is very thick and compact.

#### INVASION OF THE EPITHELIAL CELLS INTO THE MEDIAN EMINENCE

(1) The *Procellariiformes* are unique amongst birds in this respect where the tuberalis cells spread deep into the nervous tissue. This has been noted in *Diomedea melanophris*, *D. epomopphora*, *Pelecanoides magellanicus*, *Priocella antarctica*, *Procellaria acuinotialis* and *Oceanites gracilis* by Wingstrand (1951). In the *Oceanites* there was indication of such invasion only and this had not advanced much.

(2) Such invasion also takes place in some lizards (Gaupp, 1893 ; Baumgartner, 1916 ; Wingstrand, 1951 ; and Szentágothai and Székely, 1958). Wingstrand (1951) observed this picture in *Lacerta agilis* where the epithelial anlagen of pars tuberalis after losing contact with the pars distalis reach the surface of the median eminence and enter into the nervous tissue under the pial cover. These displaced cells form small islands bilaterally. Wingstrand (1951) states, "The situation of the two intracerebral cell masses in *Lacerta* on each side of the eminentia seems to correspond to the two areas of invasion in the *Procellariiformes*."

Pars tuberalis cells with Bodian-positive granules have been found in all bird pituitaries. They are, however, rare in the tuberalis of *Columba livia* and *Anser anser* (Wingstrand, 1951). He thinks that "this granulation, just as the colloid acini indicates a secretory activity of the pars tuberalis".

Cajal (1911) described the nuclei of tuber cinereum as—Noyau antérieur ou principal, Noyau postérieur ou accessoire du tuber cinereum, and Noyau supérieur. The nucleus anterior is the same as the ventromedial nucleus. The axons of the nerve cells of this nucleus proceed in the dorsal direction and the capsule is reached ; the axons proceed in anteroposterior direction. The collaterals have been very nicely described and drawn by Cajal in Figs. 312, 313 and 314. The descriptions have been given in Vol. II, page 474 to 483. According to his description the Noyau périchiasmatique ou tangentiel has got three parts—anterior, superior and posterior. The distribution is half moon-shaped, Szentágothai *et al.* (1968) mention that the anterior and superior portion of the tangential nucleus of Cajal corresponds to the true neurosecretory cells of the magnocellular supraoptic nuclei. The posterior or the retrochiasmatic part of the tangential nucleus, however, has got to be differentiated from the previous group. The large cells with the whole dendritic arborization belonging to the posterior group can be beautifully stained with the Golgi and Cox methods, whereas the neurosecretory cells of the magnocellular nuclei could not be impregnated success-



fully. The axons of the posterior group do not join the supraoptico-hypophyseal tract. The intercellular meshwork of preterminal collaterals is very much sparse in the supraoptic and paraventricular nuclei, but it is very rich in the retrochiasmatic part and characteristic synapses are seen here. The paraventricular nucleus has been depicted by Cajal (1911) in Fig. 279, page 427 as (T)—noyau sous-ventriculaire. Szentagothai *et al.* (1968) characterized tuberal neurosecretion by argyrophilic granules and granule-laden axons could be traced to the superficial layer of the median eminence and to the proximal parts of the infundibular stem (Spatz's tubero-hypophyseal system, 1951). That the fibres of hypothalamic origin could terminate in the pituitary stalk was described first by Cajal (1911) in Vol. II, page 490 of his work—"Un grand nombre des cylindres-axes destinés à l'hypophyse se ramifient déjà dans le pédicule et se terminent pres de sa surface par des extrémités variqueuses."

Szentagothai *et al.* (1968) describe the arrangement of the coarse fibred supraoptico-neurohypophyseal tract and the fine-fibred tubero-infundibular tract. The former tract originates from the large cells of the supraoptic nucleus. It is joined by fibres from the paraventricular nucleus and the conjoined fibres proceed to the posterior lobe of the pituitary. These fibres are crossed by the fine-fibred tubero-infundibular tract originating from small nerve cells situated in a halfmoon-shaped area immediately beneath the walls of the third ventricle. This is the *hypophysiotrophic area*. The fine axons of the tubero-infundibular tract end exclusively in the surface zone (zona palisadica) of the median eminence and of the most proximal part of the stalk.

Isolated medial basal hypothalamus-pituitary axis, *i.e.*, differentiated axis can operate (Halasz and Pupp, 1965). Circadian rhythm of ACTH secretion is lost in chronic experiments. This axis responds by—

- (1) intermediate or high levels of nonstress pituitary-adrenal function,
- (2) stress-response to different stressors, and
- (3) low doses of dexamethasone can suppress by feedback mechanism.

Pituitary autonomy in stressed and nonstressed female rats was studied with large medial hypothalamic lesions (MHA) by noting the levels of plasma corticosterone fluorometrically by Dunn and Critchlow (1973). Hypothalamic ablation was done by modified Halasz-Pupp knife. The modification is the presence of a horizontal cross bar (3.5 mm). So, the medial basal hypothalamus is not only isolated but there is also interruption of the vascular supply. The intact vascular supply maintains the medial basal hypothalamus. Rats with medial hypothalamic lesions did not show stress (3-min. ether) response and the corticosterone levels in afternoon nonstress condition were low. A constant low level of ACTH secretion is found after ablation of the medial hypothalamus.

The extent of the lesion in the rats was from the suprachiasmatic nucleus frontally to the premammillary or mammillary nuclei caudally. Dorsally the area extended upto the paraventricular nuclei. In all



animals arcuate and ventromedial nuclei were lesioned. There was atrophy of the neural lobes. The vascularity of the median eminence and the pituitary was intact. There was apparent hypertrophy of the intermediate lobes.

*Avian hypothalamic neurosecretory nuclei :*

These nuclear groups are mainly of two types—(a) Gomori-positive magnocellular nuclei and (b) Gomori-negative parvocellular nuclei. The supraoptic and the paraventricular nuclei comprise the Gomori-positive magnocellular nuclei. The infundibular and the ventromedial nuclei form the Gomori-negative parvocellular nuclei. Different names have been used by different authors for the supraoptic and paraventricular nuclei. The nucleus supraopticus corresponds to nucleus magnocellularis interstitialis, intermedialis, lateralis and dorsalis of Huber and Crosby (1929), nucleus magnocellularis praeopticus, medial, dorsocaudal and lateral parts of Kurotsu (1935), nucleus supraopticus of Kuhlénbeck (1937), nucleus supraopticus of Wingstrand (1951), nucleus magnocellularis praeopticus, nucleus magnocellularis supraopticus, ventrocaudal part, medial part and lateral part of Yasuda (1955), and nucleus supraopticus, ventral, anterior, internal, external, lateral and chiasmatic groups of Legait (1959). The paraventricular nucleus (Figs. 2, 3) corresponds to the nucleus magnocellularis interstitialis medialis (b) of Huber and Crosby (1929), nucleus magnocellularis periventricularis (Hauptkern) of Kurotsu (1935), nucleus paraventricularis magnocellularis (principal part) of Wingstrand (1951) and median and superoexternal groups of paraventricular nucleus of Legait (1959).

Neurosecretory cell groups were studied by Professor Wingstrand (1951) in the pigeon and in some other birds. He has given a very beautiful description of these cells, their axons and terminations. Bluishblack granular Gomori-substance more or less completely fills the neural lobe and tends to aggregate in the *glandular zones* on the surface and surrounding the vessels. The tractus hypophyseus in the fibre layer of the stem and the median eminence also contains these granules. Many nerve fibres look blue here but some appear red which take up phloxin used as a counterstain. No Gomori-positive substance has been noted in the caudal part of the glandular layer of the median eminence and this part is practically free from it but just behind the chiasma the glandular layer of the median eminence contains scattered Gomori-positive granules. The tractus supraoptico-hypophyseus on both sides can be traced to the nucleus supraopticus in the praeoptic region and to the scattered neurosecretory cells nearby. The neurosecretory fibres in the tractus hypophyseus anterior could not, however, be traced to the scattered neurosecretory cells in the anterior part of the paraventricular nucleus.

The peripheral part of the neurosecretory cells contains dark bodies and vacuoles of Gomori-positive substance are common in the cells.

Wingstrand (1951) states that the nucleus supraopticus "is situated in the praeoptic area laterally of the praeoptic recess and extends laterocaudally along the dorsal surface of the tractus opticus (Figs. 4, 5). Scat-



tered cells occur further back along the tractus supraoptico-hypophyseus through the lateral parts of the supraoptic decussation into the anterior part of the post-optic area." This nucleus is not a very compact group of cells and they spread in different directions. Some cells are exclusively neurosecretory in type whereas other parts contain smaller, *non-secretory cells*.

Neurosecretory cells are commonly found in the periventricular cell layer near the praeoptic area but Wingstrand (1951) was uncertain whether these cells should be called as the nucleus praeopticus dorso-caudalis of Kurotsu and Kuhlenbeck or to the *principal part* of the nucleus paraventricularis magnocellularis of Kurotsu. Very few neurosecretory cells were noted in the more caudal part of the latter nucleus and the accessory part of the same nucleus did not show any sign of neurosecretion. He, however, thought of the possibility of production of other kinds of neurosecretion in the *non-secretory cells*.

Avian hypothalamic neurosecretory system has also been investigated by Bargmann and Jacob (1952), Benoit and Assenmacher (1953a, b), Yasuda (1955), Stutinsky (1958), Fujita (1956), Oksche *et al.* (1959, 1963, 1964), Legait (1959), Kobayashi *et al.* (1961), Arai (1963), Farner and Oksche (1962), Oksche (1962, 1965) and others. Benoit and Assenmacher (1953, 1955) and Assenmacher (1958) have extensively studied the neurosecretory system in domestic races of the mallard. Farner *et al.* (1967) have extensively discussed the neuroendocrine mechanisms in birds.

Avian infundibular and the ventromedial nuclei comprise the Gomori-negative parvocellular nuclei. The infundibular nucleus controls the gonadotropic function.

#### THE MEDIAN EMINENCE AND THE INFUNDIBULAR STEM

In birds the *neural lobe* is always distinct and it is sharply demarcated from the *infundibular stem*. Diencephalic wall projects tubularly to the infundibular stem. The neural lobe has no connection with the pars distalis either by nerves or by vessels. The *median eminence* is the ventral or rostroventral wall of the diencephalon from the optic chiasma to the infundibular stem. It is intimately related to the primary capillary plexus of hypophyseal portal system. Professor Wingstrand (1951) described beautifully the structure of the median eminence, the neural lobe, the innervation, the vascular supply and the development of the avian pituitary. The external surface of the median eminence is smooth in most birds except in *Struthio* and *Anser* where it is deeply furrowed leading to a polylobed structure. In *Spheniscus* the entire wall is folded.

Three layers could be distinguished in a section through the median eminence—the stratum ependymale or ependymal layer, the stratum fibrosum or fibre layer, and the stratum glandulare or the glandular layer (Figs. 6, 7, 8). Nowakowski (1951) described these three layers in the cat. The outermost glandular layer was called as palisade layer by Kobayashi *et al.* (1961). Oksche (1962) distinguished two zones—zona interna consisting of ependymal layer and fibre layer, and zona externa consisting



of reticular layer and palisade layer. Aldehyde-fuchsin-positive fibres were found in the white-crowned sparrow, Zebra Finch and *C. coturnix* to leave the tractus supraoptico-hypophyseus in the anterior part of the median eminence and to turn ventrally. A dense reticular formation occurs beneath the tractus supraoptico-hypophyseus. Fine, radially directed fibres proceed from the reticulum towards the external limiting membrane where they are situated just opposite to and very near the primary capillaries of the hypophyseal portal system. The fine fibres end in the median eminence and they do not re-enter the tractus supraoptico-hypophyseus. In the posterior part of the median eminence fibres from the tractus tubero-hypophyseus cross the tractus supraoptico-hypophyseus. The fine fibres form loops in the zona palisadica and they do not contain aldehyde fuchsin positive material. So in the caudal part of the median eminence neurosecretory material is diminished in comparison to that noted in the cephalic division of the median eminence.

Benoit and Assenmacher (1953) noted five different layers in the median eminence of the duck. They are—(1) ependymal layer; (2) internal layer of the hypothalamo-hypophyseal tract; (3) layer of fine fibres derived from 2; (4) special zone with nerve loops and neurosecretory material; and (5) pars tuberalis.

The ependymal layer according to Wingstrand (1951) consists of a layer of ependymal cells lining the surface of the ventricle, very few and scattered nerve fibres and a few scattered nerve cells may occasionally be present. Processes of the ependymal cells traverse through the outer two layers to reach the external surface. Flagellated or ciliated ependymal cells may be present in some birds. Single multiciliated cells were noted among the uni-flagellated ones in *Anser*. The fibre layer is constituted by the *tractus hypophyseus* which runs from rostral or rostrolateral directions towards the infundibular stem. Processes from ependymal cells separate the bundle of fibres one from the other. Glia cells with externally disposed processes are also noted in this layer. The glandular layer is traversed by multiple fine processes of the ependymal and glia cells. These filaments run towards the external surface and are fixed to the intima piae by broad, dilated ends (*vascular feet*). The Gomori-positive substance is plenty in the fibre bundles of the tractus supraoptico-hypophyseus and in the rostral part of the glandular layer but it is absent from its caudal part. Some of the coarse fibres of the tractus hypophyseus can be seen in the glandular layer of the median eminence only in its rostral part. Fine, looped nerve fibres are noted in this zone parallel to the ependymal fibres.

### THE NEUROHYPOPHYSIS

The neurosecretory product produced in the supraoptic and paraventricular nuclei of the birds is accumulated in the median eminence and the neurohypophysis. There are four different types of neural lobes in birds.

*Type I* is noted in *Strigiformes*, *Procellariiformes*, and *Galliformes*. In these orders the neural lobe is very simple. It is formed by hollow



buds with thin primitive walls. Emigrated secondary tissue from the primitive infundibular one is very less. Only ependymal cells are present and pituicytes are rare. In adults original bilateral symmetry is lost.

*Type II*—This type is noted in majority of birds including *Struthio*, *Oceanites*, *Nycticorax*, *Charadriiformes*, *Columbiformes*, *Psittaciformes*, *Piciformes*, *Apodiformes*, and *Passeriformes*. There is a central lumen near the base of the lobe and this lumen extends bilaterally as blind diverticula which may sometimes divide into two or three diverticula each. The walls are thick and secondary tissue forms most of the gland.

*Type III*—This type is found in *Pelecanoides*, *Priocella*, *Phalacrocorax*, *Anseriformes*, *Larus*, *Perdix*, *Cuculus*, and *Caprimulgus*. The neural lobe consists of more of secondary tissue and so the organ has a compact appearance (Fig. 9). The lumen is found only at the base of the gland and on the rostro-lateral surface a pair of lateral primary diverticula are found. One or a few median diverticula may also be found. The pituicytes are plenty except in *Perdix* where the cells are few. The ependymal cells are distributed in a limited fashion.

*Type IV*—This type is seen in *Spheniscus*. The neural lobe is compact. Lobular character is lost. There are scattered membranes and connective tissue fibres within the gland. Narrow channels lined by ependyma are found in all parts of the gland. Ependymal cells and pituicytes are of equal number. Budding from the primitive sac is plenty but the buds because of fusion lose their superficial membranes. Only the connective tissue fibres are present.

The ependymal cells in the neural lobe are like those noted in the median eminence but with the difference that here the processes do not branch so much. The ependymal processes get attachment to the superficial membrane of the primitive walls or to the membrane around vessels in the secondary tissue (types III and IV) by conical vascular feet. The pituicyte has the shape of an irregular star with 2-4 processes and they are attached to the connective tissue fragments and to the intima pia around capillaries in the secondary tissue.

#### NERVE FIBRES REACHING THE MEDIAN EMINENCE AND THE NEURAL LOBE OF THE PIGEON

The description has been taken from Wingstrand (1951). All the nerve fibres at the junction between the neural lobe and the stem are concentrated in a tube-shaped tract surrounding the lumen of the stem. Towards the eminential side the tube-shaped tract continues in the fibre layer of the eminentia and then behind the optic chiasma (Fig. 7). The fibre path is known as *tractus hypophyseus* and because it can be traced to the nucleus supraopticus it is called *tractus supraoptico-hypophyseus*. The *tractus hypophyseus posterior* is formed by few coarse fibres which separate from the *tractus hypophyseus* just at the base of the stem and turn upwards along the posterior surface of the hypothalamus. *Superficial eminentia plexus* is constituted outside the fibre layer in the median emi-



nence and in the proximal parts of the stem by a diffuse plexus of delicate and irregular fibres (Fig. 7c). This plexus is mainly formed by *tractus tubero-hypophyseus* coming from the tuber nuclei. Numerous fibres with an irregular course end in this plexus and there are also transverse fibres with decussation. This plexus innervates the glandular layer. The peculiar loops in the glandular layer (Figs. 6, 7) take their origin exclusively from this plexus. The course of the *tractus tubero-hypophyseus* before joining the superficial eminentia plexus is very peculiar. From the tuber nuclei (Fig. 10) some fibres proceed along the lateral surfaces of the hypothalamus and enter the eminentia close to the intima piaie and situated outside the *tractus hypophyseus*. Some other fibres take a separate course along the ventricle and reach the ependymal layer inside the *tractus hypophyseus*. These fibres run ventromedially and then laterally passing between the bundles of the *tractus perpendicularis* to the surface and join the superficial plexus (Figs. 7, 11). In the anterior third of the eminentia the superficial plexus is still present and it cannot be separately distinguished from the fibre layer as the two lie very close to each other and the glandular layer receives some fibres from the *tractus hypophyseus* and these fibres form the peculiar loops in the glandular layer. The superficial plexus in this part gets also plenty of fibres from *tubero-hypophyseal tract*. Postchiasmatic crossed and uncrossed fibres form the *tractus supraoptico-hypophyseus* (Fig. 7B). The *tractus hypophyseus anterior* is formed by all postchiasmatic hypophyseal fibres which do not belong to the *tractus supraoptico-hypophyseus* or the *tractus tubero-hypophyseus* (Fig. 7A).

The *tractus hypophyseus posterior* arises from a diffuse nucleus situated ventral to the *decussatio tractus infundibuli* (Figs. 4, 11) and is called *nucleus subdecussationis* by Wingstrand (1951). The *tractus tubero-hypophyseus* is formed by fibres coming from the tuberal nucleus which is same as Kuhlenbeck's nucleus tuberis and Huber and Crosby's nucleus hypothalamicus inferior + nucleus mamillaris medialis ventralis and Rendahl's *nucleus m* (Figs. 4, 11). The *tractus supraoptico-hypophyseus* arises from the supraoptic nucleus. The fibres of the *tractus hypophyseus anterior* can be traced to the lateral and inferior hypothalamic nuclei, and Kuhlenbeck's n. paraventricularis posterior hypothalami, n. paraventricularis magnocellularis and n. praepreticus paraventricularis (Figs. 4 : D, 12).

#### ELECTRON MICROSCOPIC STRUCTURE OF THE AVIAN MEDIAN EMINENCE AND THE NEURAL LOBE

Kobayashi *et al.* (1961) examined the median eminence of the parakeet, *Melopsittacus undulatus* and noted the processes of ependymal cells, neuroglial cells and terminal parts of axons with four types of structures. The processes of ependymal cells could be identified by the presence of large number of fibrillar structures. At the terminal segments of axon paths there were structures with average diameters of 390 Å (synaptic vesicles), other larger ovoid vesicles with diameters of



490 A, elementary neurosecretory granules (600 to 1000 A) and vesicles of the same size as the elementary granules. Structures which are intermediate between synaptic vesicles and ovoid vesicles could be found. Presence of all or some of these structures could be found in different nerve terminations. Folds of thick basement membrane of the primary capillaries of the hypophyseal portal system enter into the median eminence and axon endings with synaptic and larger vesicles terminate on the membrane. Processes of ependymal and neuroglial cells interpose between the nerve terminations and the basement membrane. Typical elementary granules and vesicular structures are found in the fibre layer of the median eminence. Processes of ependymal fibres and sections of nerves have also been noted. Oota and Kobayashi (1962) and Bern and Nishioka (1965) studied the ultrastructure of the avian median eminence.

Duncan (1956), Legait and Legait (1958), and Kobayashi *et al.* (1961) noted the terminations of the neurosecretory axons in the neural lobe. These terminations are surrounded by pituicytic cytoplasmic processes. The terminations contained the same structures noted in the median eminence. The granules vary in size from 600 to 1000 A in the terminations located in the median eminence but the diameter ranges from 600 to 1750 A in the neural lobe.

Oksche (1965), Oehmke *et al.* (1969) and Oksche *et al.* (1970) studied the hypothalamo-hypophyseal system of birds with the help of electron microscope. They found in *Passer domesticus* that the granules (diameters up to 1000 A) of the tubero-infundibular tract are formed in the Golgi zone of the cells of the infundibular nucleus. Axodendritic and axosomatic synapses are found in the nerve cells of the infundibular nucleus. In the nerve endings of the median eminence there are different types of structures. They contain vesicles (400-600 A), vesicles (400 A) and granules (800 A), granules (800-1000 A), vesicles (300-400 A) and granules (1200 A) and granules from 1200 A to 1600 A. In the pars nervosa of the hypophysis the diameter of the granules is from 2000 A to 2500 A.

Farner *et al.* (1967) in their survey on neuroendocrine mechanisms in birds stressed that, "A very important question is that of possible functional synaptic contacts between neurosecretory and Gomori-negative fibres either in the reticular layer or in the peripheral part of the palisade layer." This type of functional relationship was raised in the past by many authors.

Calas and Assenmacher (1970) studied the ultrastructure of the median eminence in the canard (*Anas platyrhynchos*). Within the subependymal layer there were neurons of the nucleus infundibularis, axons and varicosities with dense core granules, and capillaries with structural characteristics similar to those in the superficial layer. The axons of the hypothalamo-neuro-hypophyseal tract were seen in the fibre layer. There was evidence for at least three main types of neurosecretory axons in the palisade layer. Peczely and Calas (1970) described the ultrastructure of the median eminence in the pigeon (*Columba livia domestica*) in differ-



ent experimental conditions. Hypothalamic control of adrenocorticotrophic function was specially discussed with particular reference to the role of the 1200-1400 Å granules.

#### ENZYMATIC ACTIVITY IN THE MEDIAN EMINENCE AND THE NEURAL LOBE

Kobayashi *et al.* (1962) found increase in acid phosphatase activity in the neural lobe of dehydrated pigeons without any increased activity in the median eminence. Similar feature was noted in the White-crowned sparrow by Farner *et al.* (1964) and Kawashima *et al.* (1964). Similarly, increased acid phosphatase activity has been noted in the median eminence without any change in the neural lobe after photostimulation of testicular growth in the White-crowned sparrow by Kobayashi and Farner (1960), Farner *et al.* (1964) and in *Zonotrichia albicollis* by Wolfson and Kobayashi (1962). This proves a dissociated response in between the median eminence and the neural lobe and that an acid phosphatase may be responsible for the release of neurohormones into the primary capillaries of hypophyseoportal vessels.

Photoperiodic gonadal stimulation in *Zonotrichia leucophrys gambelii* (the White-crowned sparrow) led to an increase in catheptic proteinase activity in the median eminence, whereas Kobayashi *et al.* (1962) did not find any such change in the neural lobe. This is also an example of the dissociated response; but dehydration increases such activity in the neural lobe and the median eminence. Increased activity in the neural lobe is associated with the increased release of antidiuretic hormone. Increased activity at the median eminence is due to the concomitant release of corticotropin-releasing factor (CRF) as Kawashima *et al.* (1964) could find that there was depletion of sudanophilic material in the interrenal cells after dehydration.

Acetylcholinesterase activity was found in the median eminence of the White-crowned sparrow by Kobayashi and Farner (1964). Uemura (1964) noted this activity in *Zosterops palpebrosa japonica*. Kobayashi (1965) found it in *Passer montanus*. AChE activity is associated with aldehyde-fuchsin-positive and aldehyde-fuchsin-negative endings. The activity is weaker in the neural lobe. Cholinergic mechanisms may help in the release of releasing factors.

Kobayashi (1964) said that monoamine oxidase (MAO) activity in the neurosecretory system of the tree sparrow was studied by Matsui in his laboratory. Moderate MAO activity was seen in the AF-positive neurosecretory cells. Reaction in the pars nervosa was slight. MAO activity was noted in the median eminence surrounding the small blood vessels or primary capillaries of the hypophyseoportal system. Little or no reaction was found in the supraopticohypophyseal tract and the ependymal cells. The cytoplasm of the glia cells gave a strong reaction. MAO activity in the median eminence is located mainly in the aldehyde-fuchsin-negative nerve endings and/or the processes of the glia cells. As catecholamines are not present in the glia cells, MAO is present only at the terminations of the aldehyde-fuchsin-negative fibres of the median



eminence (posterior part). Thus adrenergic mechanisms may help in the release of releasing factors into the primary capillaries of the hypophyseal portal system of the median eminence.

### AVIAN HYPOTHALAMIC MONOAMINES

Avian hypothalamic monoamines have been demonstrated by Fuxe and Ljunggren (1965), Bjorklund *et al.* (1968), Sharp and Follett (1968), Oehmke *et al.* (1969), Sharp and Follett (1970), Oksche *et al.* (1970) and Oksche (1971) with histochemical fluorescent technique.

Fuxe and Ljunggren (1965) studied the cellular localization of monoamines in the upper brain stem of the pigeon. Green and yellow fluorescence developed indicating the presence of a primary catecholamine and 5-hydroxytryptamine respectively. Low concentration of amines is found in the cell bodies and axons but very high concentration is found in the terminals, specially in the abundant varicosities. Three large ascending monoamine systems are present within the upper brain stem. The fibres start from cell bodies located within the mesencephalon and run towards mainly in the medial forebrain bundle. Two of the neuron systems produce and store a primary catecholamine, one probably giving rise mainly to terminals within the hypothalamus and the preoptic area, the other within the corpus striatum. The remaining system produces and stores 5-hydroxytryptamine.

In the median eminence (palisade layer) fluorescent terminals are small in number though they are variable. Sharp and Follett (1970) state that the fluorescent terminals in the palisade layer are probably derived from the monoamine containing axons in the subependymal layer. Bjorklund *et al.* (1968) found stronger fluorescence of the chicken than in the pigeon. Sharp and Follett (1968) observed similar terminals in the anterior and posterior division of the quail median eminence. The terminals terminate on the primary capillary plexus. Sharp and Follett (1970) state that in the reticular layer fluorescent structures are few but there are some coarse beaded fibres which run from cephalic to caudal direction.

Fluorescent nerve terminals were noted in the *nucleus tuberis* and the *nucleus hypothalamicus posterior medialis*. Sharp and Follett (1970) found catecholamine-containing nerves to proceed from *nucleus tuberis* to the subependymal layer after coursing around the base of the third ventricle and so this forms a part of the tubero-hypophyseal tract in the quail. Fibres from the *nucleus hypothalamicus posterior medialis* may also add to the previous fibre system.

Fluorescent cell bodies have not yet been well detected in the avian hypothalamus although in the mammalian *nucleus tuberis* catecholamine containing cell bodies are seen well in pregnancy and lactation.

The *nucleus hypothalamicus posterior medialis* is placed in such a central location for reception, modulation or transmission of information that message can easily be conveyed by adrenergic pathways between



higher brain centres, anterior hypothalamus or lower brain stem and the nucleus tuberis or median eminence. Sharp and Follett (1970) found fluorescent tracts to proceed in the stratum cellulare internum connecting with the anterior hypothalamus while lateral tracts join the forebrain bundle.

In the anterior hypothalamus the supraoptic and paraventricular nuclei are surrounded by monoaminergic nerve fibres. Plexus of adrenergic fibres is more in the medial division of the supraoptic nucleus than in the lateral division. The adrenergic fibres of this system come from cells located in the pons and medulla.

Dopaminergic neuronal system starting in the hindbrain and terminating in the nucleus basalis and the forebrain after passing through the forebrain bundle also occurs in the avian brain.

Monoaminergic supraopticohypophyseal system also occurs in birds. The fibres come from the medial division of the supraoptic nucleus.

Oksche (1971) summarized the observations regarding detection and localization of avian neurones producing neurohormones and releasers with special reference to the hypothalamo-hypophyseal system. The anterior division of the median eminence is Gomori-positive. The same positive reaction is noted in the supraoptic and paraventricular nuclei and in the neurosecretory pathway leading to the neural lobe. With Falck-Hillarp fluorescence preparation, arcuate (infundibular) nucleus with fluorescent structures is seen but the neural lobe gives a complete negative picture. Amongst and in between fluorescent and non-fluorescent structures of the hypothalamus there are some other types of cells which should be considered as *releasers*. In the White-crowned sparrow the anterior group of portal vessels is connected with the anterior median eminence and the cephalic lobe of the anterior pituitary and posterior group connects the posterior median eminence with the caudal lobe. The anterior median eminence is Gomori-positive whereas the posterior median eminence is Gomori-negative. Lesion near the optic chiasma interrupts the supply of Gomori-positive substance to the anterior median eminence. Bern and Nishioka (1965) noted in the house sparrow that the elementary granules in the neural lobe are bigger than those noted in the Gomori-positive area. Oksche (1967) and Oehmke *et al.* (1969) observed in the house sparrow that the supraoptico and paraventriculo-hypophyseal tracts contain the elementary granule with a diameter of 200 nm but the largest granule has got a diameter of 120-150 nm in the anterior median eminence. He suggests that the anterior hypothalamus contains the cells from where these aldehyde-fuchsin-positive fibres come to the anterior median eminence. Amongst the cells of the supraoptic nucleus having 200 nm granules there were some cells with smaller (120-150 nm) granules. These correspond to those smaller granules noted in the anterior median eminence (Gomori-positive cells). He further says that "There are also some fluorescent elements in the proximal neurosecretory pathway, so that this part of the tract does not contain only nerve fibres connecting the anterior hypothalamus



with the neural lobe but also other fibres, fluorescent or non-fluorescent, connecting some anterior nuclei of the hypothalamus with the ME."

Boissin and Assenmacher (1971) discussed the implication of the central aminergic mechanisms regarding the determination of the circadian rhythm of blood corticosterone in the quail.

Peczley (1971) studied the effect of metyrapone, prednisolone and insulin treatment on the domestic pigeon's hypothalamus. There is a close functional correlation between the aldehyde-fuchsin-positive neurosecretory system of the anterior hypothalamus and the median eminence and the corticotropic activity. The arcuate nucleus may play an important part in the regulation of ACTH secretion.

Bouille and Bayle (1973) conducted experimental studies on the adrenocorticotrophic area in pigeon hypothalamus. In the posterior medial and lateral hypothalamic areas there is a well-defined adrenocorticotrophic area destruction of which leads to the same decrease of the plasma corticosterone level as is noted after adenohipophysectomy and this also prevents the progressive recovery of adrenocortical function after autografting.

Calas (1973) studied the monoaminergic innervation of the median eminence in the canard (duck) *Anas platyrhynchos* including radioautographic and pharmacologic studies. Findings suggest that the neurones of the median eminence are probably modulated by catecholamines and other neurotransmitters on their dendrites and on their soma.

#### CELL TYPES OF THE AVIAN PARS DISTALIS

The cell types have been described by Rahn and Painter (1941), Wingstrand (1951, 1963), Mikami (1958, 1960), Tixier-Vidal *et al.* (1962), Benoit (1962) and Tixier-Vidal (1963). The avian adenohipophysis has no intermedia, the pars tuberalis is constantly present and the pars distalis is divided into a cephalic lobe and a caudal lobe.

Benoit (1962) described the histology of the duck's pituitary. Acidophilic cells are alpha and eta and the glycoproteidic cells are beta, gamma, and delta cells. There are three types of gonadotropic cells. Gamma cell which is one type of gonadotropic cell is found only in the caudal lobe of the pituitary. It is carminophilic in azan stain, purple in Herlant's tetrachrome, light blue in Methasol blue—PAS method and it is PAS positive. Period of activity is from December to April (testicular growth period). The cells involute with regression of testes. These cells produce LH.

Cephalic lobe beta cells are not carminophilic. They are PAS positive and purple with Herlant's dye. With Methasol blue—PAS these cells look violet blue. These cells are active from February to July. They secrete FSH. The eta cells are found in the cephalic lobe. They are erythrosinophilic in Herlant's dye, PAS negative, and look light blue with Methasol blue—PAS. They are active from December to April. Probably they secrete LTH.



Seven cell types have been demonstrated in the anterior hypophysis of the duck by Tixier-Vidal (1963). They are the three mucoprotein forms: beta, delta and gamma and three acidophilic forms: alpha, eta and epsilon. The seventh type called kappa is peculiar to birds. Alpha and gamma cells are found in the caudal lobe and the beta, eta, epsilon and kappa cells are located in the cephalic lobe. The delta cells are equidistributed between the two lobes. Identification of FSH-beta cells, LH-gamma cells, prolactin-eta cells and TSH-delta cells could be done. Corticotropic activity seemed to be localized in the epsilon acidophilic cells. STH could be secreted by alpha cells and kappa cells are the source of MSH.

Farner *et al.* (1967) said that the cells of the cephalic lobe are controlled primarily by neurohormones secreted by supraoptic and paraventricular nuclei and the cells of the caudal lobe are controlled by neurohormones from the infundibular nuclei.

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## Chapter 17 (A)

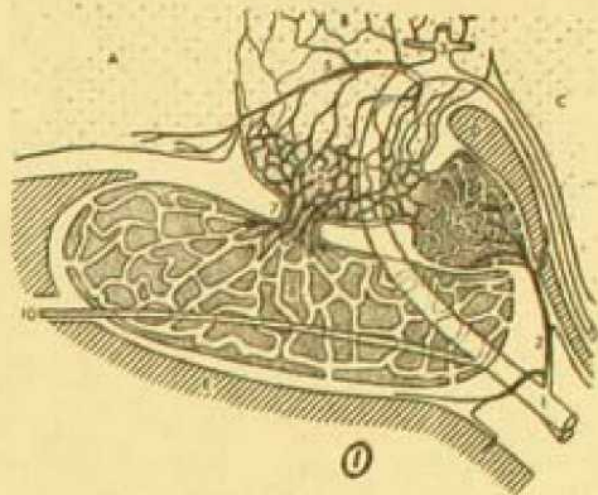


Fig. 1. Diagram of the vessels of the pituitary of *Columba livia*.

A—chiasma opticum, B—diencephalon, C—medulla oblongata, D—dorsum sellae, E—osseous floor of the sella.

1—arteria carotis interna, 2—a. hypophyseae inferior, 3—anterior ramus, and 4—posterior ramus of the a. carotis, 5—a. infundibularis, 6—primary capillary plexus on the eminentia, 7—portal vessels, 8—secondary plexus in the pars distalis, 9—capillary bed of the neural lobe, 10—a. ophthalmica interna.

From a thick median section through the sphenoid region of a pigeon injected with ink and cleared in benzyl benzoate. The capillary nets are simplified, and the perihypophyseal veins are not considered. (Wingstrand, 1951). (Courtesy of Professor K. G. Wingstrand).



Fig. 2. Transverse section through the paraventricular nucleus of the goose. (Trichrome stain,  $\times 100$ ).





Fig. 3. Transverse section through the paraventricular nucleus of the goose. To the left is the ependymal lining and to the right the nerve fibres. (Kluver-Barrera stain.  $\times 100$ ).

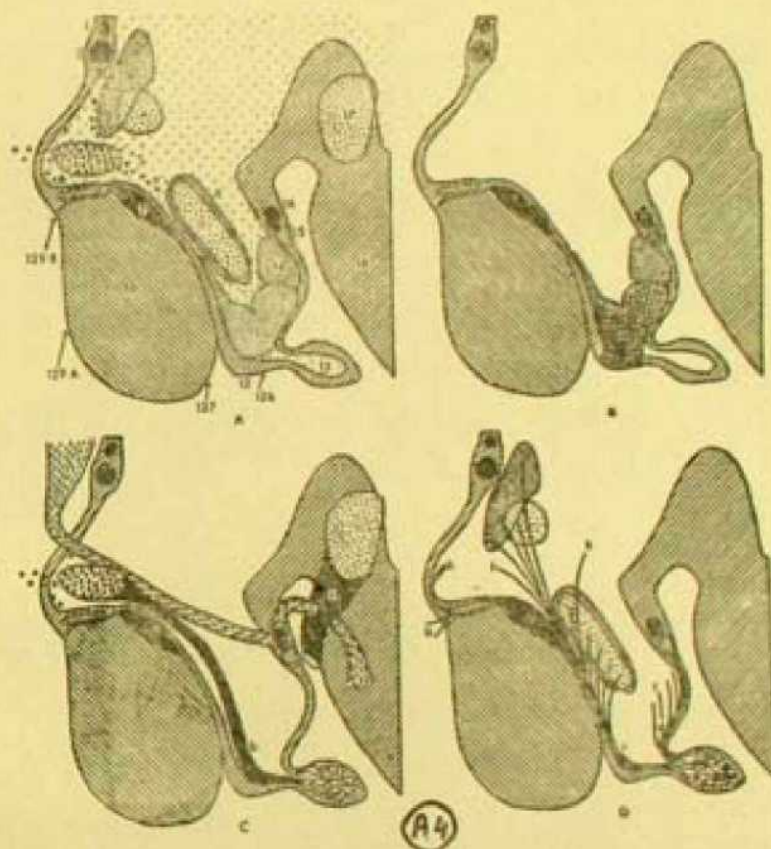


Fig. 4. Diagrams showing the innervation of the avian neurohypophysis. Fig. A. shows the situation of the nuclei, fig. B. the tractus tubero-hypophyseus, fig. C. the tr. supraoptico-hypophyseus, fig. D. the tr. hypophyseus anterior and posterior.

Explanation to Fig. A : 1—commissura pallii, 2—commissura anterior, 3—principal and 4—accessory part of the n. supraopticus, 5—n. supraopticus, 6—recessus praesopticus with the median praesopticus nucleus in its ventral wall, 7—supraoptic decussations, 8—n. inferior hypothalami, 9—n. lateralis hypothalami, 10—chiasma, 11—n. tuberis, 12—eminencia mediana, 13—neural lobe, 14—n. mammillaris, 15—n. subdecussationis, 16—decussatio tractus infundibuli, 17—nuclei of nervous oculomotorius (simplified), 18—medulla oblongata.

The arrows (126, 127, 129 A, B) indicate the planes of figures 11, 12, and 5.

Explanation to Fig. B-D : a—tr. tubero-hypophyseus, b—tr. supraoptico-hypophyseus, c—tr. hypophyseus anterior, d—tr. hyp. posterior, e—tr. infundibuli, —nervous oculomotorius, g—autonomic fibres from fasciculus opticus, h—"fasciculus supraopticus" (Rhatig), i—fibres, probably from praesopticus areas, k—fibres, probably from paraventricular areas, l—fibres from cells near the surface of the posterior hypothalamus. (Wingstrand, 1951). (Courtesy of Professor K. G. Wingstrand).



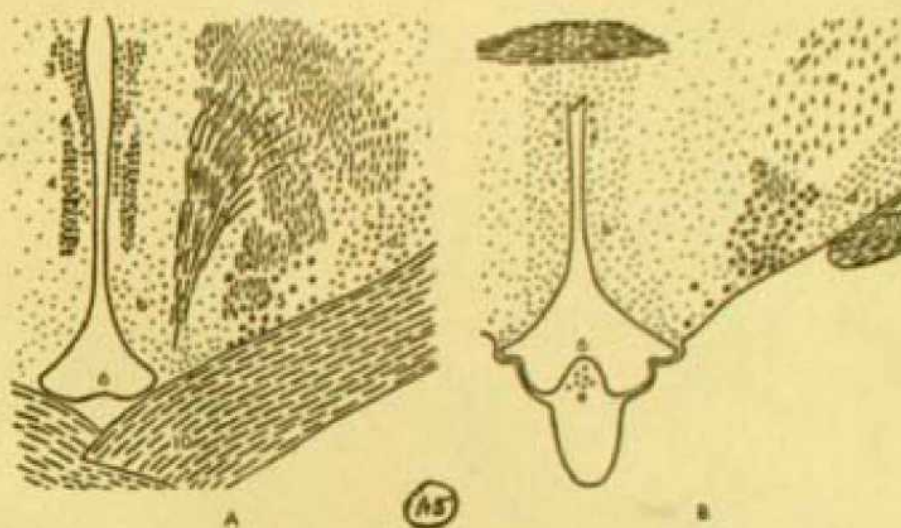


Fig. 5. *Columba livia*, ad. Cross section through the praectopic region.  
 A—immediately in front of the supraoptic decussations (plane 129A), B—through the commissura anterior (plane 129B). The planes 129A, B are indicated in Fig. 4A.  
 2—commissura anterior, 3—principal and 4—accessory part of the nucleus paraventricularis magnocellularis, 5—n. supraopticus, 6—recessus praectopicus, 7—decussatio supraoptica dorsalis, 10—chiasma and tractus opticus.  
 a—forebrain bundles, b—praectopic periventricular nuclear masses, c—tractus infundibuli, d—nucleus corresponding to the n. ventrolateralis in the chick (Kuhlenbeck), e—median praectopic nucleus. Camera lucida drawings of 10 $\mu$  sections, fixed in Bouin and stained in Gomori's haematoxylin and phloxin. Nerve tracts only approximately indicated. 35  $\times$ . (Wingstrand, 1951). (Courtesy of Professor K. G. Wingstrand).



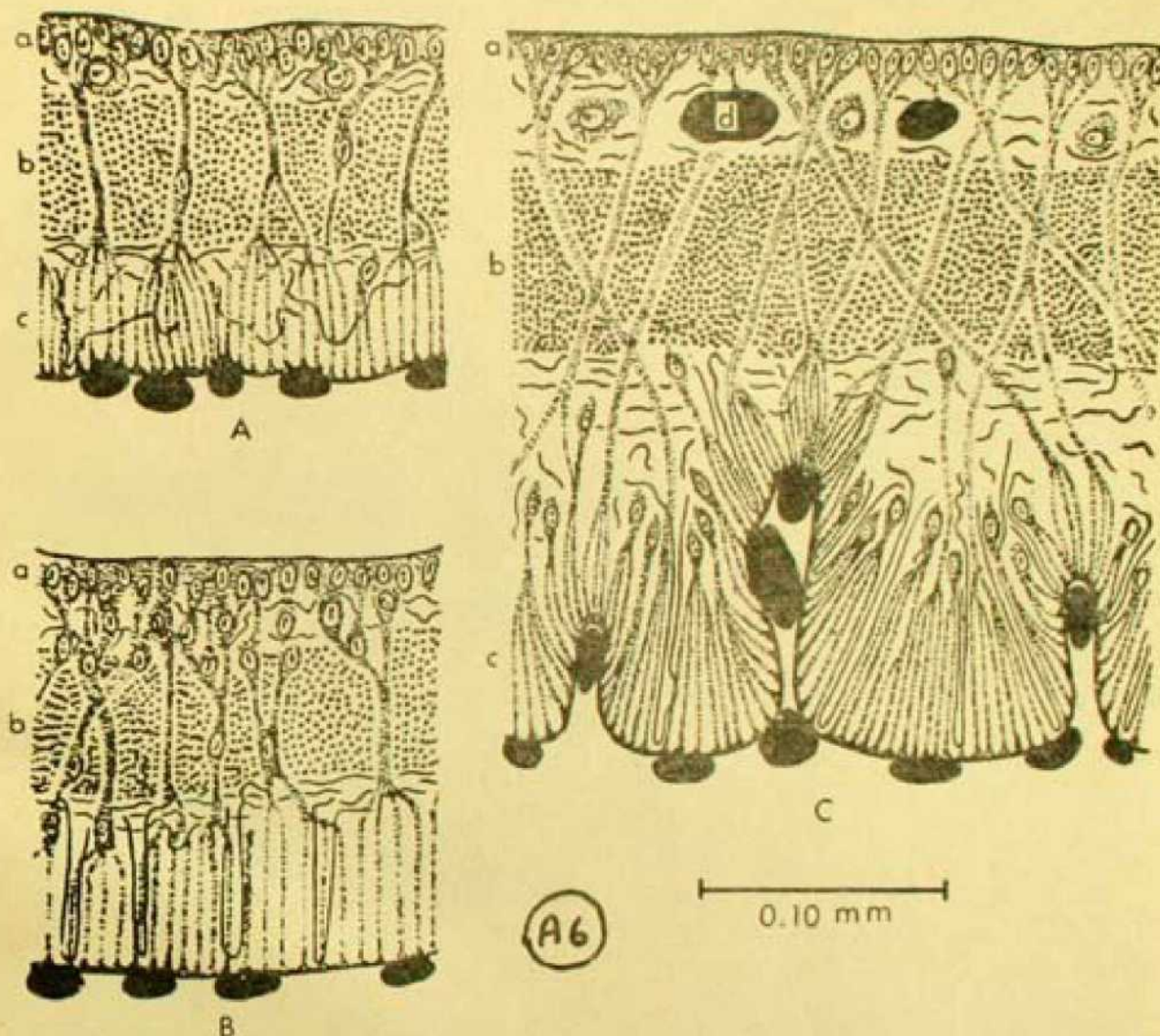


Fig. 6. Diagrams showing a cross section of the eminentia mediana in *Coragyps atratus* (A), *Columba livia* (B), and *Anser anser* (C). Ependymal and glia cells are stippled, blood vessels are black, and nerve fibres are indicated by black lines, or in cross section, by large dots.

a—ependymal layer, b—fibre layer, c—glandular layer, d—non-portal, hypothalamic capillaries. A and C from  $8\mu$  sections, fixed in Bouin and stained in Bodian-azan. B is a synthesis of several slides. The magnification is the same for all figures (note scale to the right). (From Wingstrand, 1951). (Courtesy of Professor K. G. Wingstrand).



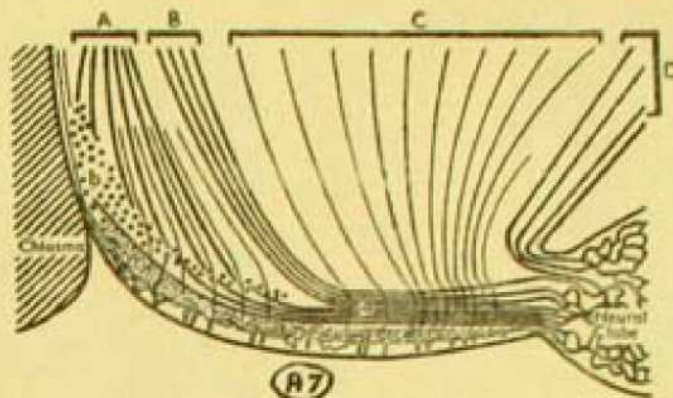


Fig. 7. Diagram showing the behaviour of the nerve fibres in the neurohypophysis of the pigeon (*Columba livia*).

A—tractus hypophyseus anterior, B—tr. supraoptico-hypophyseus, C—tr. tubero hypophyseus, D—tr. hypophyseus posterior.  
a—fibre layer of the eminentia (tr. hypophyseus),  
b—decussation of the tr. hypophyseus anterior,  
c—decussation of the superficial eminentia plexus, which is indicated with delicate lines.

(Courtesy of Professor K. G. Wingstrand).

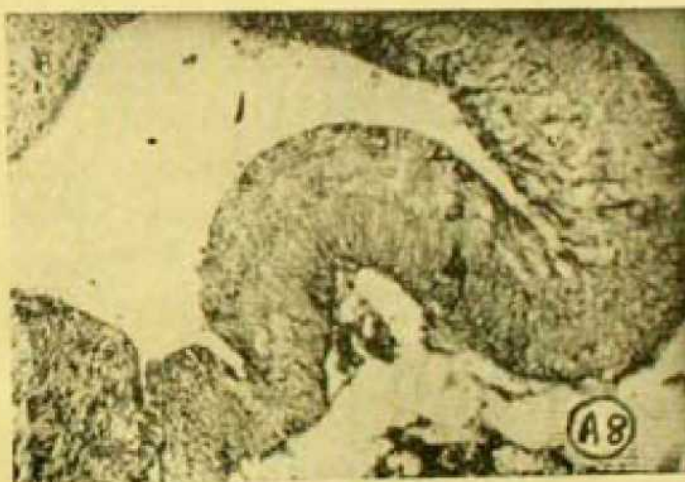


Fig. 8. Median eminence of the goose showing the three layers :

1—ventricle ; 2—ependymal layer ; 3—fibre layer ;  
4—glandular layer.

(Trichrome stain,  $\times 100$ ).



Fig. 9. Compact neural lobe of the goose (Type III).  
Plenty of pituicytes. (Trichrome stain,  $\times 100$ ).



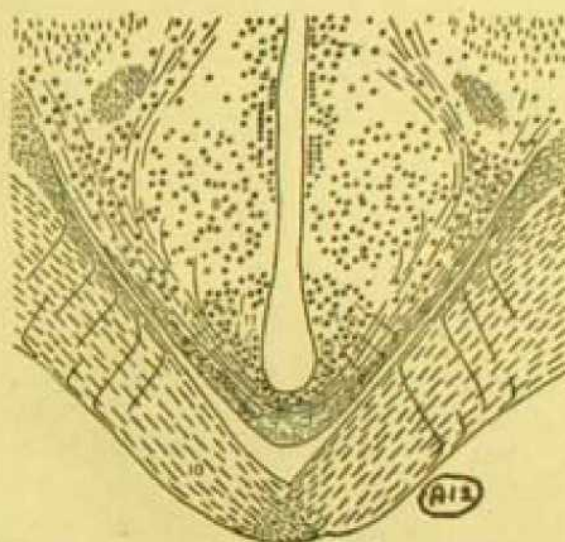
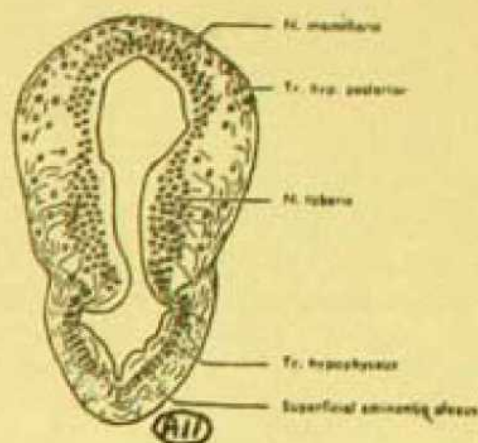
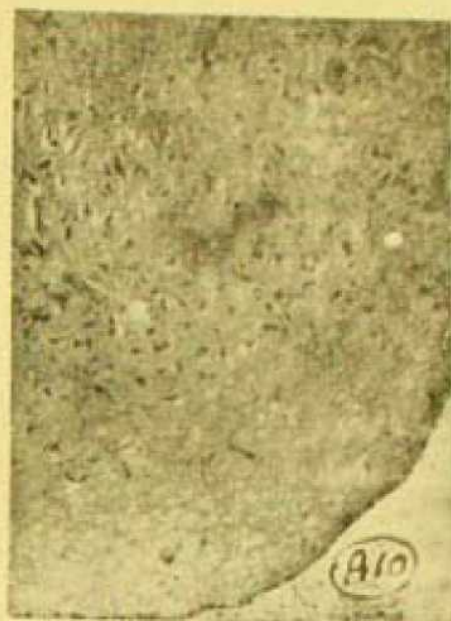


Fig. 10. Transverse section of the ventral hypothalamus of a goose showing tuberal cells. (Trichrome stain.  $\times 100$ ).

Fig. 11. *Columba livia*, ad. Cross section through the posterior part of the hypothalamus corresponding to plane 126 in Fig. 4A. Alcohol-formalin-acetic acid, paraffin  $15\mu$ , impregnation with silver according to Palmgren.  $35\times$ . (Wingstrand, 1951). (Courtesy of Professor K. G. Wingstrand).

Fig. 12. *Columba livia*, ad. Transversal section through the rostral part of the eminentia (the plane 127 is indicated in Fig. 4A). 8—n. inferior hypothalami, 9—n. lateralis hypothalami, 10—chiasma, 11—n. tuberis.

a—ramus basalis caudalis of tr. septomesencephalicus, b—tr. infundibuli, c—medial fibres of tr. hypoph. anterior, probably from periventricular thalamic areas, d—n. paraventricularis posterior, e—tr. opticus basalis (Frey, 1937), f—tr. hypoph. anterior, g—tr. supraoptico-hypophyseus, h—superficial eminentia plexus. Camera lucida drawing. Fibres and cells are much more numerous in the original. Alcohol-formalin-acetic acid, paraffin  $15\mu$ , silver impregnation according to Palmgren.  $35\times$ . (Wingstrand, 1951). (Courtesy of Professor K. G. Wingstrand).



## BRAIN MECHANISMS RESPONSIBLE FOR ACTH RELEASE IN THE PIGEON (COLUMBA LIVIA)

de Roos (1963) reviewed the physiology of the avian interrenal gland. It is stated that corticosterone and aldosterone are the major corticoid hormones secreted by the avian interrenal. The interrenal is dependent on the anterior pituitary for normal function but there is a great deal of controversy regarding the degree of such control. Even in the absence of the pituitary, the interrenal functions at a high level (Miller and Riddle, 1942 ; Miller, 1961 ; Assenmacher, 1958 ; Benoit, 1962 ; Ma and Nalbandov, 1963). Farner *et al.* (1967) conclude that the avian adrenocorticotrophic activity of the pars distalis is only partially regulated by hypothalamic neuroendocrine mechanisms. Semi-independent function of the adrenal cortex is possible in the absence of the pars distalis. Péczley (1971) and Bouillé and Baylé (1973) indicated the existence of hypothalamic adrenocorticotrophic area in the pigeon. Salem *et al.* (1970) had indications that chicken hypothalami contain ACTH or an ACTH-like substance. They also indicated that the adrenal ascorbic acid depletion in hypophysectomized rats by chicken hypothalamic extracts was not due to vasotocin. The hypothalamic depleting substance was different from hypophysial ACTH. Corticotropin releasing factor (CRF) is present in the chicken hypothalami.

### OBSERVATIONS

- (1) *Feedback receptor sites in the median eminence region of the pigeon sensitive to adrenocortical steroids :*

Corticosterone, cortisol and dexamethasone have been implanted into the basal region of the hypothalamus (median eminence), dorsal hypothalamus and pituitary. The animals were killed by decapitation one week after operation. Adrenal weight, plasma and adrenal corticosterone levels were analysed. There was diminished adrenal weight, and diminution of plasma and adrenal corticosterone levels when dexamethasone was put in the median eminence. Cortisol and corticosterone implanted into the median eminence had similar results except the fact that they could not change the adrenal weight. Dorsal hypothalamic and anterior pituitary implantation had no effect. The responses were more in the dexamethasone group.

There was a fall of 50% of plasma corticosterone level when ACTH was implanted into the median eminence without any change in the adrenal weight whatsoever (three days after the implantation).

- (2) *Corticotropin releasing factor (CRF) after destruction of supraoptic, paraventricular, tuberal and ventromedial nuclei and anterior median eminence of the pigeon (Figs. 1, 2).*

These lesions (electrolytic) when singly done could not reduce the CRF of the median eminence ; rather it was high on some occasions. It



proves thereby that CRF producing neurones are not localised to any of the particular areas concerned. Maximum increase was observed in the group where the anterior median eminence was damaged. Plasma corticosterone levels were measured after intracarotid injection of the hypothalamic extracts [prepared after the method of Vernikos-Danellis (1964)] in dexamethasone blocked (median eminence) pigeons.

(3) *CRF in the median eminence and plasma of the hypophyseoportal vessels of the pigeon :*

CRF has been found in the median eminence. There is a rise in the CRF content after the stress of ether anaesthesia for 45 minutes or fracture (1 day). The hypophyseoportal vessels have been approached through the transorbital route and divided. Blood collection rate varied between 0.4 ml. and 0.5 ml./hour. Plasma corticosterone levels were measured after intracarotid injection of the portal vessel plasma in dexamethasone blocked (median eminence) pigeons. Presence of CRF in the portal vessel plasma has been noted in the pigeon. CRF in the pigeons' portal vessel plasma diminished in animals with implantation of corticosterone, cortisol and dexamethasone in the basal region of the hypothalamus (median eminence). Plasma from peripheral vessels did not contain CRF activity to any great extent as compared to the results obtained by injection of portal vessel plasma.

(4) *Aldehyde-fuchsin-positive material and plasma corticosterone level in normal and stressed (fracture) pigeons :*

Aldehyde-fuchsin-positive material

	Supraoptic nucleus.	Paraventricular nucleus	Anterior median eminence	Posterior median eminence	Neural lobe.	Rise in plasma corticosterone level.*
Normal pigeon (5)	+	+	+	0	+	
Stress (fracture) of right femur (3rd Hr.) (7).	Partial depletion in some animals.	0	Partial depletion not detected in all animals.		0	91%
Stress (fracture) of right femur (1 day) (8).	Depletion in more number of animals.	0	Depletion in more number of animals.	0	0	100%

+—Present ; 0—No change ; ( )—Number of animals.

\* After the method of Guillemin *et al.* (1959), modification of Silber *et al.* (1958).



(5) *Changes in epsilon cells of the anterior pituitary, and adrenal in response to fracture and dexamethasone injection in the pigeon :*

Types of treatment	Epsilon cells (ACTH producing cells)	Adrenal
Normal	These cells are located in the cephalic lobe of the pituitary and start from the junction of cephalic and caudal lobe and spread throughout the cephalic lobe. Herlant's tetrachrome stains them purple rose. These are small round cells. Sometimes the granulations are very faint and vacuolations occur. The nucleus has got a nucleolus.	Functional zonation is possible. The subcapsular zone produces aldosterone. The deeper layers produce corticosterone and are controlled by ACTH.
Fracture of right femur. (5 to 7 days).	There was increased number of these cells. The granulations could easily be detected. (Fig. 3).	Stimulation (fig. 4).
Dexamethasone (1 mg./day for 6 days).	The activity of these cells was below normal and they were less in number and atrophic.	Atrophic conditions.

(6) *Adenohypophyseal grafting in the medial basal hypothalamus of the pigeon and other areas—cellular morphology.*

Location of the graft	Cellular morphology		(Cellular morphology) Stress response to fracture (3-5 days).
	Herlant's tetrachrome	Alcian blue —PAS-orange G	
(1) Upper eye lid (Fig. 5).	Cells were small in size. There was diminished cytoplasm and the nuclei were also small.		No change could be seen.
(2) Dorsal hypothalamus (basal aspect) (Figs. 6, 7).	Do.		Do.
(3) Ventral hypothalamus (median eminence, tuberal nucleus, ventromedial nucleus and retrochiasmatic area adjoining the aldehyde-fuchsin-positive fibre-area) (Figs. 7, 8, 9, 10).	Active granulated cell types could be seen. PAS-positive basophils and blue delta basophils could be identified. The grafts also contained eta and epsilon cells.		Increased activity in the epsilon cells and the basophils could be observed.

(7) *Stimulation and lesion experiments of the brain in the pigeon and ACTH release :*

Freely moving birds with chronically implanted electrodes of 0.3 mm. in diameter with insulation except at the tip were used for stimulation experiments. These were bilaterally implanted either superficially or at a depth. In the control series no stimulation was applied. The



frequency of stimulation was 15–30 cps, the duration of the rectangular pulse was 3 msec, the voltage used was from 3 to 5 V. The total time of stimulation varied from 3 to 8 minutes.

In the lesion experiments, surface lesions were produced surgically and depth-lesions were produced by electrocautery.

Peripheral blood corticosterone levels were studied 1/2 hour after stimulation and in the lesion experiments after 3 to 5 days.

Stimulation experiments (Figs. 11, 12, 13, 14)	Release of ACTH	
(1) Ventral and posterior hypothalamic stimulation—median eminence, nucleus hypothalamicus posteromedialis, nucleus inferior and nucleus arcuatus.	Increased ACTH release	
(2) Archistriatal stimulation	Increased ACTH release	
(3) Hippocampal, septal and septomesencephalic tract.	Diminished ACTH release	
Lesion experiments (Figs. 15, 16)	ACTH Release	Stress of fracture and ACTH release.
Hippocampus, septum, septomesencephalic tract and medial forebrain bundle.	Increased ACTH release.	Increased ACTH release

Areas influencing ACTH release in the brains of the fish, the toad (*Bufo melanostictus*) (Figs. 17–24), the reptile and the mammals have also been mentioned by Roy.

### SUMMARY AND CONCLUSION

(1) There are feedback receptor sites in the median eminence region of the pigeon (*Columba livia*) which are sensitive to adrenocortical steroids.

(2) Corticotropin releasing factor (CRF) is present in the median eminence. CRF producing neurones are not localized to any particular ventral hypothalamic nuclear groups, rather a widespread nuclear chain is involved in this process.

(3) Stress of ether anaesthesia or fracture leads to a rise in CRF content of the median eminence. The hypophyseoportal vessel plasma contains CRF.

(4) Evidences of correlation have been observed between the aldehyde-fuchsin-positive material in the supraoptic nucleus, anterior median eminence and plasma corticosterone level. Stress responses have been observed.



(5) Epsilon cells in the cephalic lobe of the pars distalis of *Columba livia* produce and secrete ACTH. Stress of fracture stimulates the pituitary-adrenal-axis and dexamethasone inhibits it.

(6) Hypophysiotropic area (median eminence, tuberal nucleus, ventromedial nucleus and retrochiasmatic area adjoining the aldehyde-fuchsin-positive area) could be identified in *Columba livia* from pituitary grafting (in medial basal hypothalamus) experiments. Stress response could be observed in the epsilon cells and basophils of the grafted pituitaries.

(7) Stimulation and lesion experiments of the brain of *Columba livia* have been conducted with a view to note the control of ACTH release. Areas from where increased ACTH release has been obtained on stimulation are ventral and posterior hypothalamus (median eminence, nucleus hypothalamicus posteromedialis, nucleus inferior and arcuate nucleus), and archistriatum. Hippocampus, septum, septomesencephalic tract and medial forebrain bundle are inhibitory areas for ACTH release.

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## Chapter—17

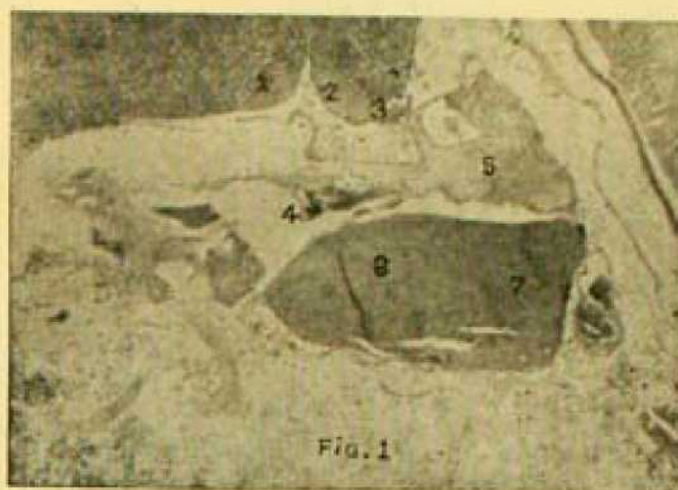


FIG. 1



FIG. 6

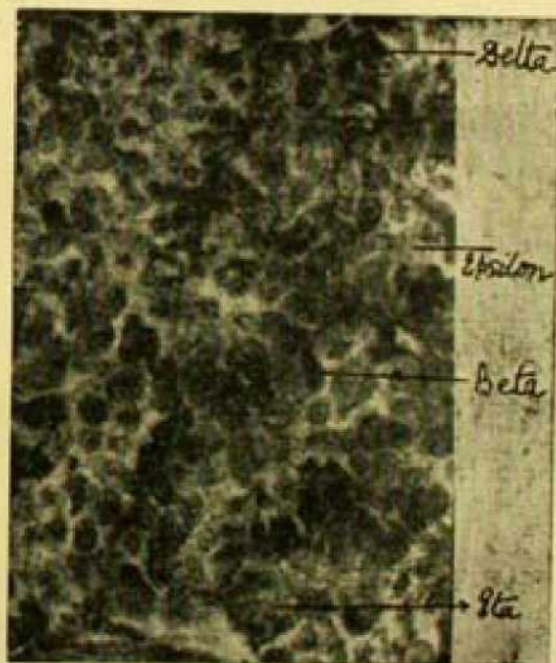


FIG. 3.

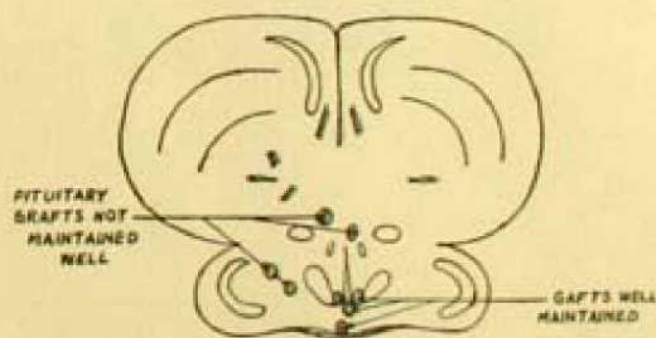


FIG. 7. SHOWING POSITION OF  
PITUITARY GRAFTS IN THE  
HYPOTHALAMUS OF THE PIGEON

FIG. 7

- Fig. 1. Sagittal section of the brain and pituitary of *Columba livia*. Haematoxylin and eosin stain. 1—optic chiasma. 2—anterior median eminence. 3—posterior median eminence. 4—hypophyseoportal vessels. 5—neural lobe. 6—cephalic lobe of pars distalis. 7—caudal lobe of pars distalis.
- Fig. 3. Increased activity of the epsilon cells in the cephalic lobe after stress. Herlant's tetrachrome stain.  $\times 400$ .
- Fig. 6. Pituitary graft in the dorsal hypothalamus. Herlant's tetrachrome stain.  $\times 400$ .
- Fig. 7. Showing position of pituitary grafts in the hypothalamus of the pigeon.



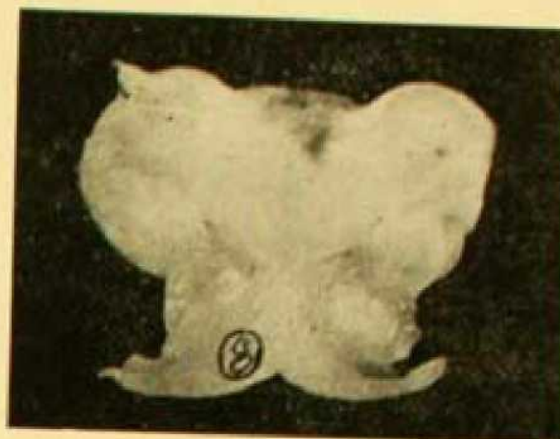


FIG. 8



FIG. 9



FIG. 10

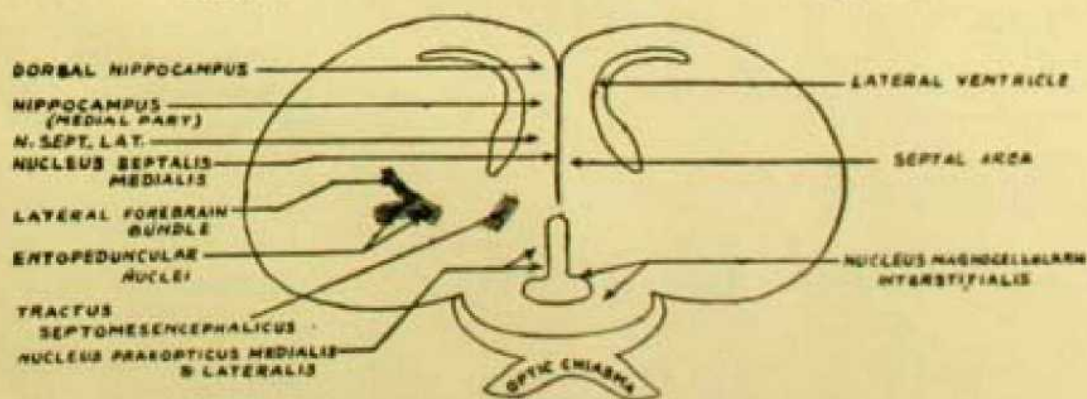


FIG. 11

**TR SECTION OF FOREBRAIN OF THE PIGEON THROUGH  
OPTIC CHIASHMA**

- Fig. 8. Transverse section of the pigeon's brain to show the location of the active pituitary graft in hypophysiotropic area.
- Fig. 9. Well Vascularized pituitary graft in the medial basal hypothalamus.
- Fig. 10. An active portion of the pituitary graft in Fig. 9 showing differentiated cell types which are well granulated and there are PAS-positive basophils.  $\times 400$ .
- Fig. 11. Transverse section of forebrain of the pigeon through optic chiasma.



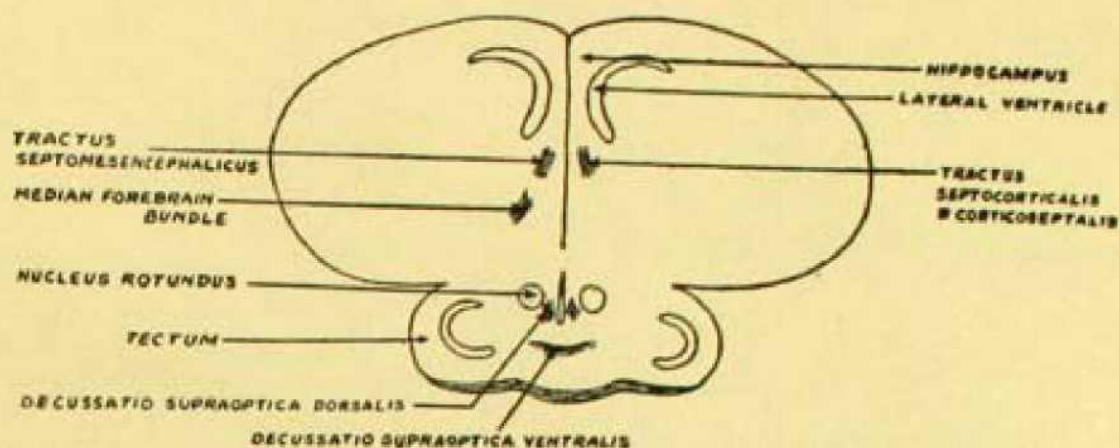


FIG. 12

**TR. SECTION OF THE PIGEON'S BRAIN THROUGH  
NUCLEUS ROTUNDUS**

Fig. 12. Transverse section of the pigeon's brain through nucleus rotundus.

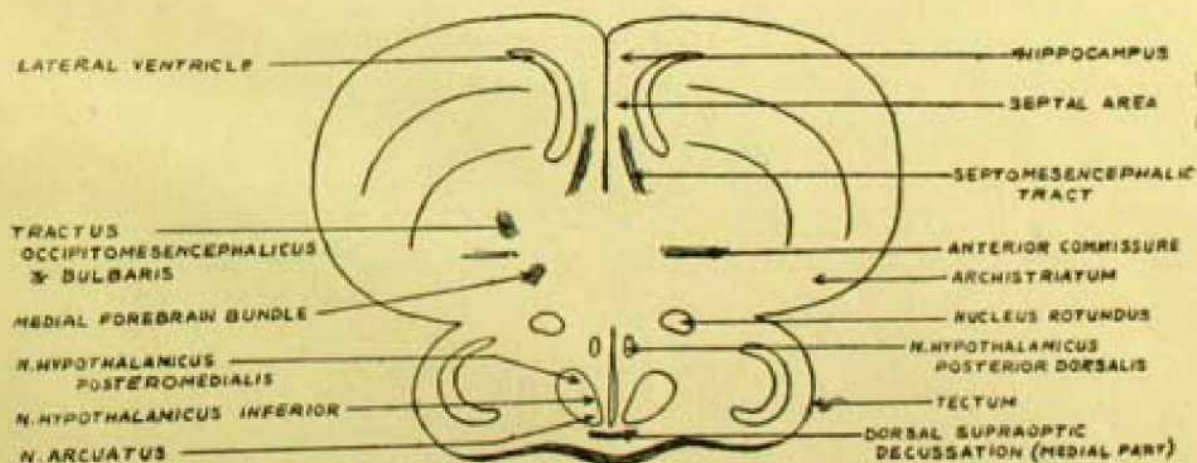


FIG. 13

**TR. SECTION OF THE PIGEON'S BRAIN JUST POSTERIOR  
TO ANTERIOR COMMISSURE TO NOTE THE  
POSITION OF THE HYPOTHALAMIC NUCLEI**

Fig. 13. Transverse section of the pigeon's brain just posterior to anterior commissure to note the position of the hypothalamic nuclei.



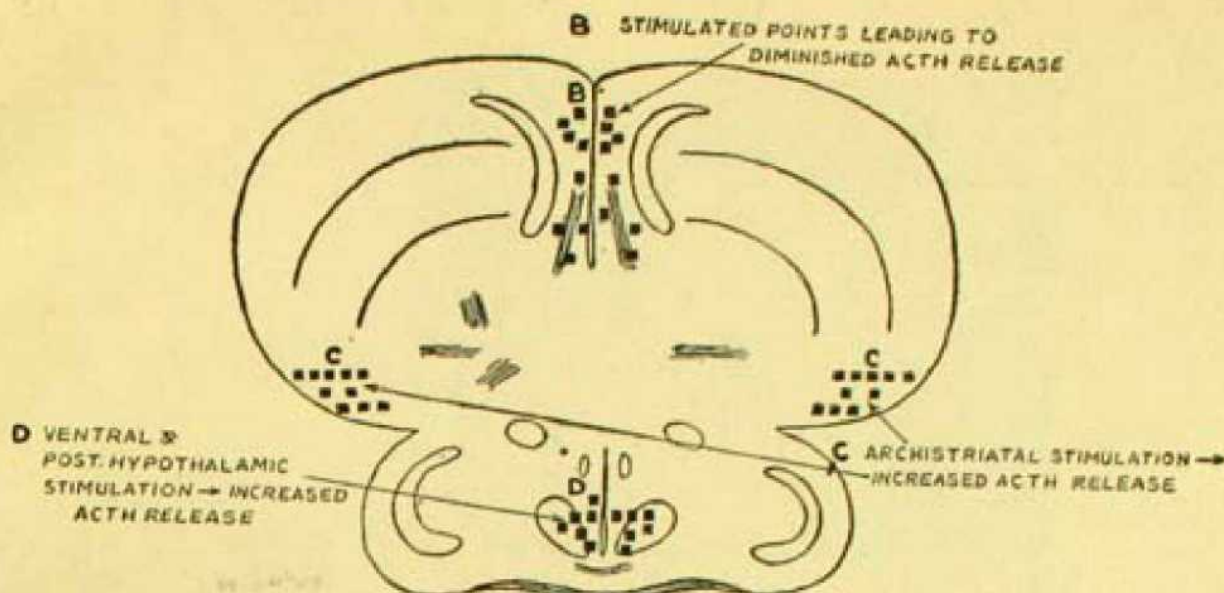


FIG. 14

**TR. SECTION OF THE PIGEON'S BRAIN JUST POSTERIOR TO ANTERIOR COMMISSURE TO NOTE THE POSITION OF THE HYPOTHALAMIC NUCLEI & POINTS OF STIMULATION**

Fig. 14. Shows the positions of the tips of the stimulating electrodes.

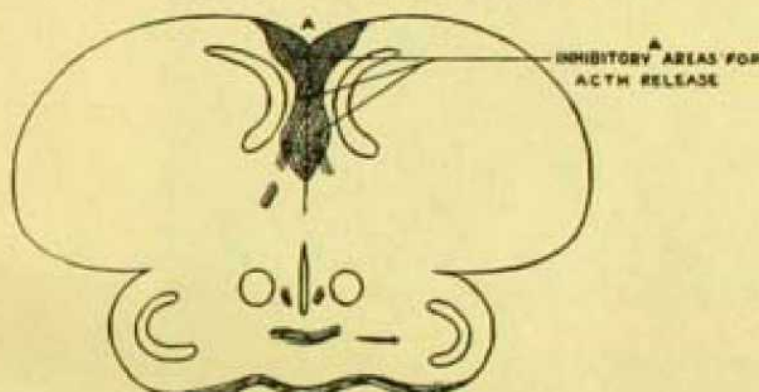


Fig. 15

**TR. SECTION OF THE PIGEON'S BRAIN THROUGH NUCLEUS ROTUNDUS LESION EXPERIMENTS**

Fig. 15. Shows inhibitory areas for ACTH release. There is increased ACTH release after lesion of these areas.

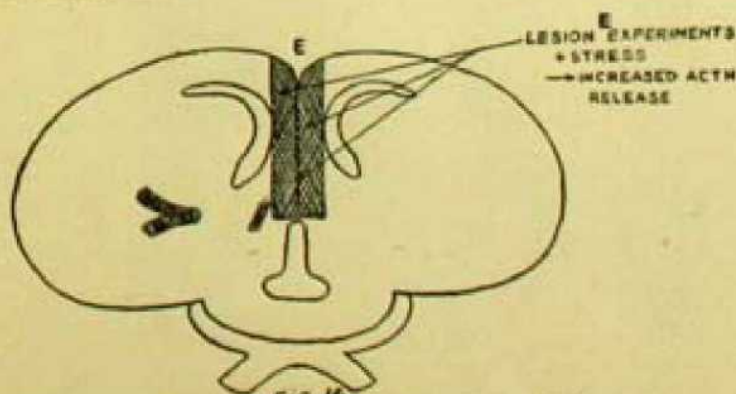
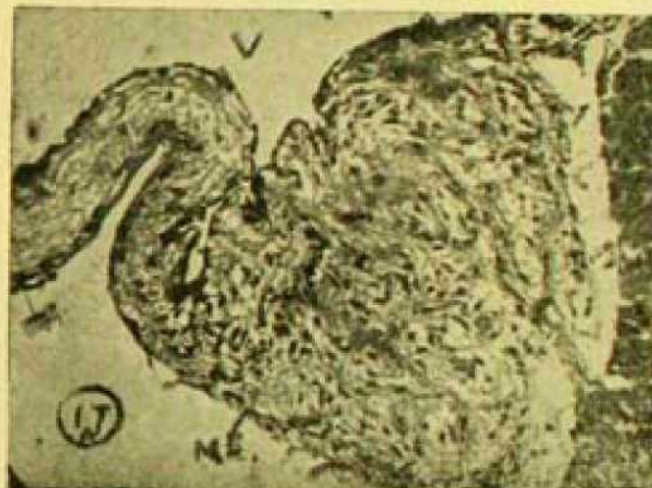
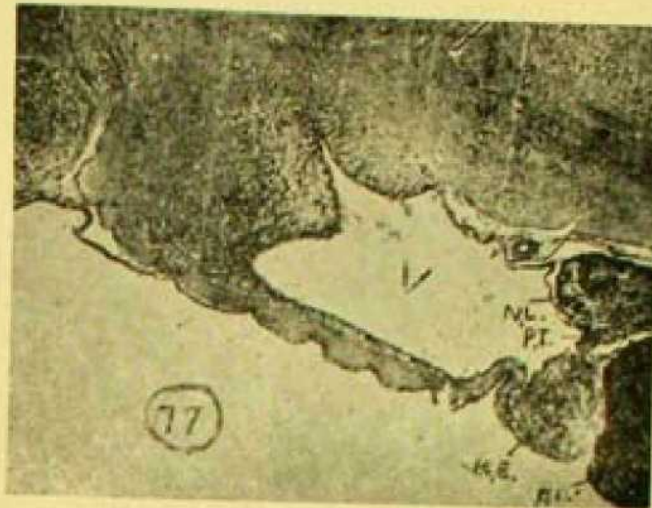


FIG. 16

**TR. SECTION OF FOREBRAIN OF THE PIGEON THROUGH OPTIC CHIASMA LESION EXPERIMENTS + STRESS**

Fig. 16. Lesion experiments and stress lead to increased ACTH release.





Figs. 17 & 17A. Sagittal section of the median eminence (ME), neural lobe (NL), pars intermedia (PI) and pars distalis (PD) of *Bufo melanostictus*. V=ventricle.

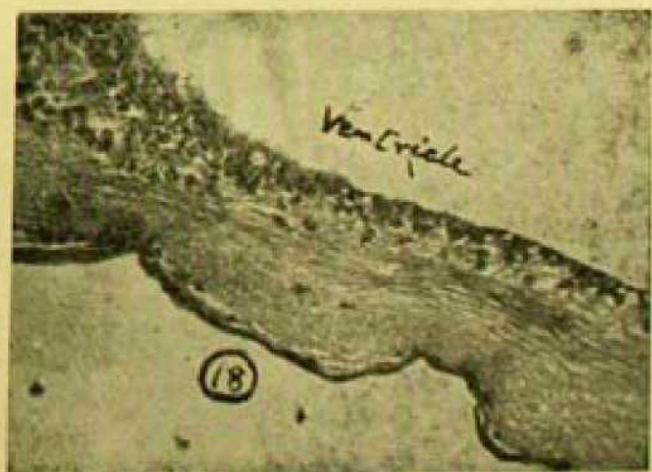


Fig. 18. Sagittal section of the floor of the infundibular recess of *Bufo* to show ependymal layer, the fibre layer and the outer layer (enlargement of portion of Fig. 17.).





Fig. 19. Horizontal section of the preoptic nucleus of *Bufo* showing the bipolar neurones having two processes—HP=hypophyseal process, VP=ventricular process, E=ependymal lining. Aldehyde fuchsin.

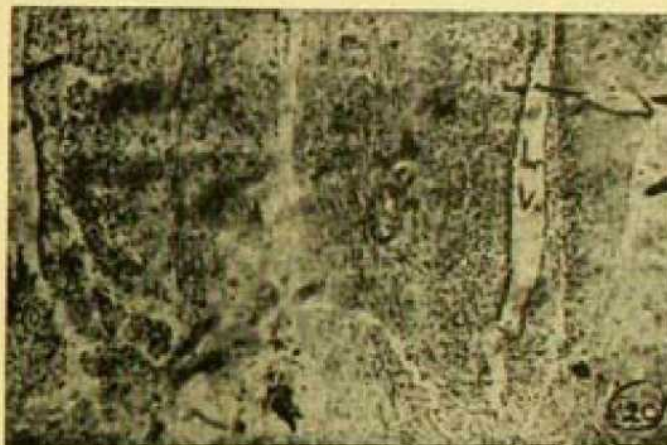
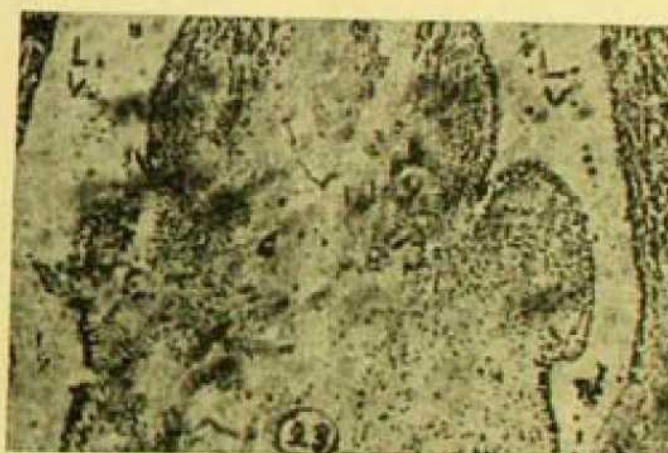


Fig. 20. Medial forebrain bundle of *Bufo*. LV=lateral ventricle.



Fig. 21. Medial forebrain bundle of *Bufo* at higher magnification.





- Fig. 22. Medial and lateral forebrain bundles of *Bufo* (Horizontal section). MFB=medial forebrain bundle ; DEC. MFB—decussation of medial forebrain bundle.
- Fig. 23. Horizontal section of the brain of *Bufo*. Hip=hippocampus ; SEP=medial and lateral septal area ; LV=lateral ventricle.
- Fig. 24. Horizontal section of the brain of *Bufo* showing optic chiasma. Opt. tr.=optic tract ; Inf.=infundibulum.



## CHAPTER 18

### ADDENDUM

#### THE CYTOLOGY OF THE PITUITARY GLAND OF THE TELEOST (EEL) AFTER PROF. M. OLIVEREAU

OliverEAU (1976) in a personal communication states that regarding the source of ACTH, in teleost fishes (Fig. 1), it is neither in acidophilic nor in basophilic cells—an obsolete terminology. ACTH is secreted by peptidic cells, but they do not stain with blue or red dyes; they can be demonstrated with the alizarin blue which gives a purple colour, and with the lead-haematoxylin which gives a dark blue-black colour. In non-teleosts, their localization seems to be rostral, but no typical staining reaction has yet been determined.

OliverEAU studied the role of prolactin in osmoregulation and stimulation of prolactin cells when dopaminergic fibres are destroyed or inhibited and reduction of activity of the same cells when dopaminergic control is stimulated.

Fontaine and OliverEAU (1975) briefly reviewed the anatomical structure of the pituitary from hagfish to birds. Peculiar features of chondrichthyans are the ventral lobe of the selachians and the pharyngeal pituitary of holocephalans. They have suggested a homology between them. Initially the contact between adeno and neurohypophysis is very slight or absent and it reaches a maximum in teleosts, where the hypothalamo-hypophysial portal system is rarely detected. Well-developed portal system has been noted in higher vertebrates where the contact is limited to the intermediate lobe. There are seven to eight categories of adeno-hypophysial cells. The functions of these cells have been studied by staining reactions and their responses to various stimuli and inhibitions. The functional interpretation of them, has now often been verified by immunochemistry and immunofluorescence. Contradictory statements, however, occasionally appear with the use of these techniques. Fontaine and OliverEAU think that they may be partially related to the zoological specificity of various hormones, to the presence of subunits in the glycoprotein hormones or to multiple states of the same hormone. There exists circannual rhythms for gonadotrophic and thyrotrophic functions. Pituitary circadian rhythms have been noted in relation to circulating hormone level. The few data can be attributed only with reserve to specific cellular rhythms and the subject needs further investigation.

OliverEAU and Dimovska (1969) identified the cell types in the auto-transplanted pituitary gland under the dorsal skin of the eel. The prolactin secreting cells could easily be identified. They had large round nuclei, big nucleoli and showed almost complete degranulation in 14 out of 15 animals. The corticotrophic cells could be detected along the ramifications of the nervous tissue and these cells were more or less granulated.



They showed a subnormal activity. The thyrotrophic cells were angular and well granulated. Their number was less compared to that in the *in situ* pituitary. They could not be found out in a few cells. The growth hormone—secreting orangeophilic cells were numerous having dense granulations and variable degrees of activity. Amongst these somatotrophic cells there were chromophobic cells which probably corresponded to the gonadotrophic cells which are always poorly differentiated in the control eels.

The pars intermedia cells appeared to be quite active and granulated. The neurohypophysis was atrophic having some fibrocytes and numerous pituicytes. The infundibular recess with ependymal layer could be well visualized. There was no neurosecretory material. The graft was well vascularized.

In the eel only the gonadotrophic cells and one cell type (PAS-positive) of the pars intermedia are completely hypothalamic dependent for their function. Some autonomous activity could be seen in the other cell types when the pituitary loses its connexion with the hypothalamus. The autonomy in the eel seems to be more for the somatotrophic and prolactin cells and perhaps also for the corticotrophic cells than in *Poecilia*. In *Poecilia* the thyrotrophic function is better preserved than in the eel. Pars intermedia of the eel secretes some intermedin.

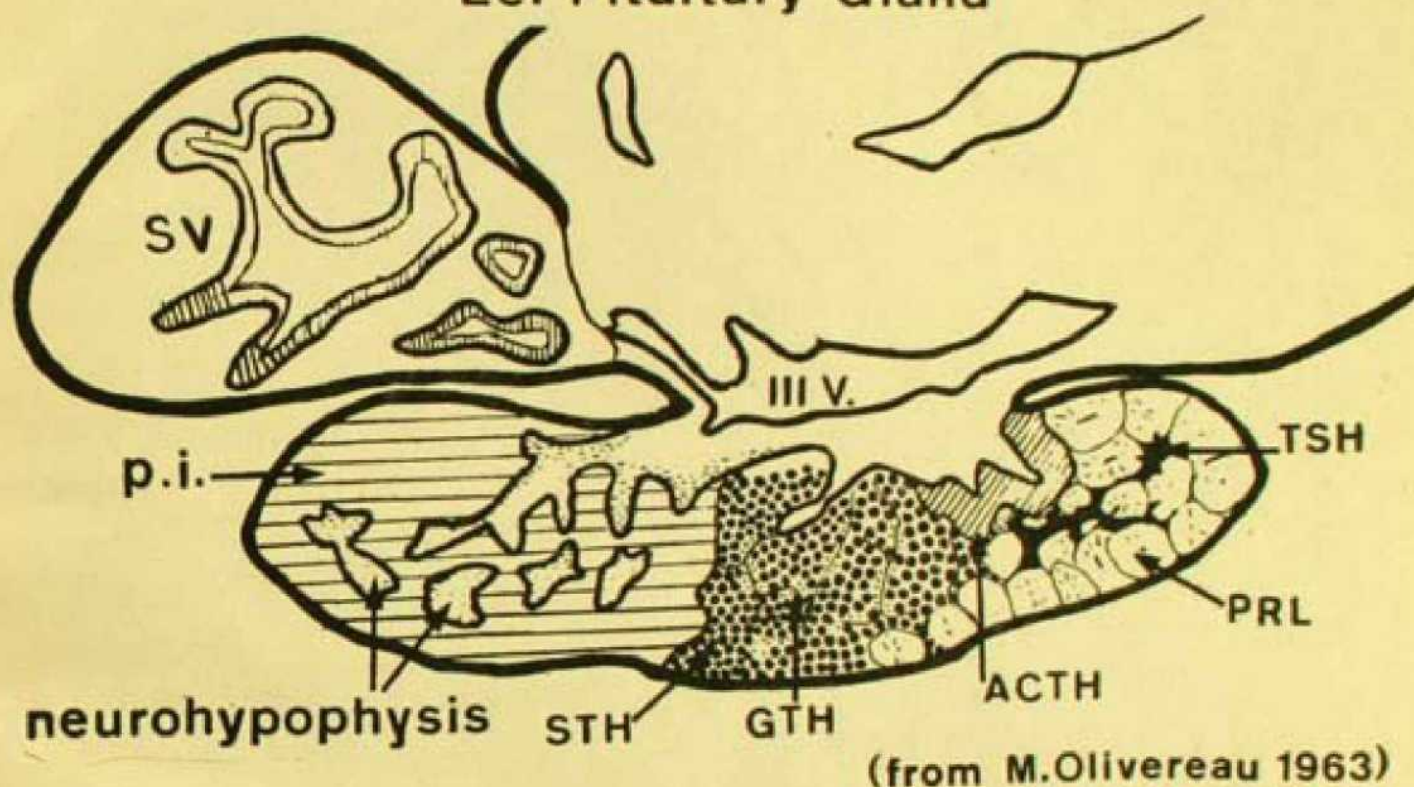
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## Chapter—18

### Eel Pituitary Gland



LEADS TO THE FIGURE

- Fig. 1.—The pituitary gland of the eel.
- P. i. — Pars intermedia
  - STH — Somatotrophic cells or growth hormone secreting cells
  - GTH — Gonadotrophic cells
  - ACTH — Corticotrophic cells
  - PIL — Prolactin cells
  - TSH — Thyrotrophic cells
  - S. V. — Saccus vasculosus
  - III V — Third ventricle.

(Through the courtesy of Professor M. Olivereau and the Academic Press, General and Comparative Endocrinology.)



## APPENDIX

Chapters XV, XVI, XVII and XVIII comprised the Lady Brahmachari Readership Lectures delivered by the author on 10th, 11th and 13th of November, 1975 in Dr. Bidhan Chandra Roy Post-graduate Institute of Basic Medical Sciences, University College of Medicine, Calcutta University. The lecture series was inaugurated by Sri A. K. Panja, Bar-at-Law, Hon'ble Minister-in-Charge of Health and Family Planning, Govt. of West Bengal, and was presided over by Prof. Dr. P. K. Bose, M. Sc., D. Phil., F. S. S., Pro-Vice-Chancellor (Academic), University of Calcutta.





## LADY BRAHMACHARI READERSHIP LECTURE SERIES OF 1975

*Inauguration by—*

Sri AJIT KUMAR PANJA, Barrister-at-Law,  
Minister-in-Charge, Department of Health & Family Planning,  
Government of West Bengal  
on 10th November, 1975

I feel honoured to have this unique opportunity to inaugurate Lady Brahmachari Readership Lecture Series of 1975 (Calcutta University) to be delivered by Dr. Binod Bihari Roy, Head of the Department of Surgery and Experimental Surgery of R. G. Kar Medical College and Hospitals, Calcutta. Dr. Roy is also the Head of the Department of Experimental Surgery of the University College of Medicine, Calcutta University. He graduated from the R. G. Kar Medical College in 1947 and successively obtained M. S. (Surgery), Ph. D. (Physiology), D. Sc. (Physiology), D. Sc. (Experimental Surgery) from the Calcutta University. He is also F. N. A. He was as brilliant a student as he is now as a teacher and a researcher ; he was Class-Assistant in Surgery, and Mohendra Datta Scholar in Clinical Surgery. In the University, he stood first in Surgery and was awarded McLeod and Pashupatinath Medals. Later, in the year 1963, in appreciation of his original contribution for the advancement of Medical Sciences, Calcutta University awarded Coates Gold Medal to Dr. Roy. His greatest achievement is the organization of the Department of Experimental Surgery at the R. G. Kar Medical College. In fact he is pioneer in this field in our country. His special fascination is Neuro-endocrinology in the area of which Dr. Roy has been working upon relentlessly for more than quarter of a century and has published a number of outstanding papers in different forums in this country and abroad. The present series of lectures is, in fact, built upon his vast experience as a teacher and researcher in this complicated field of Medical Science. I presume that the contents of these lectures will be subsequently published by Calcutta University and I am sure that the book will be of immense benefit to the future generation of students as well as the advanced research workers and surgeons.

As a Surgeon he has earned a great reputation and his skill and operative technique has been of value towards amelioration of the sufferings of orthopaedic cripples. Dr. Roy as a Teacher and Surgeon has beautifully organised the Department of Surgery at R. G. Kar Medical College & Hospitals and it is for his leadership the Surgeons of his institution could rise to the occasion to give relief to the victims of last Ultadanga Railway accidents. Prompt and relentless services rendered by the team under guidance of Dr. Roy in this Railway accident will remain ever green in the minds of all who have witnessed the situation.

Before I conclude, I must heartily congratulate Dr. Roy for his exemplary and industrious research achievements for which all of us feel proud. He has come far, he will go further.

I am thankful to the authorities of Calcutta University for extending me this invitation.

A. K. PANJA





*Address by the President : Prof. P. K. Bose, M. Sc., D.Phil., F.S.S. (Lond.), F.S.I. (Ind.), F.I.A. (Eng.), Pro-Vice-Chancellor for Academic Affairs, Calcutta University.*

Professor Binod Bihari Roy is one of the few Medical men in our country who has a passion for research. He is at present the Head of the Department of Experimental Surgery at the Calcutta University. His special field of interest is Neuro-endocrinology. In this area he has been working for more than 25 years ; his contributions were published in the important journals in our country and abroad. He was instrumental in setting up the Department of Experimental Surgery at the R. G. Kar Medical College, Calcutta. He is the recipient of a large number of prizes and medals awarded by the Calcutta University. At present he is trying to build up a school round him. He is the author of a number of books.

The country looks forward to Dr. Roy for more important contributions in his field of research.

SENATE HOUSE

P. K. BOSE

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